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JULY, 1930

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Physiological Zoölogy

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THE EFFECT OF CYANIDE ON THE CARDIAC RHYTHM OF EMBRYOS OF FUNDULUS HETEROCLITUS

(Three figures)

FLOYD J. BRINLEY

Physiological Laboratory, Battle Creek College, and Marine Biological Laboratory, Woods Hole

IT HAS been frequently impossible in studying the physiological effects of sub-lethal concentrations of cyanide, to distinctly separate the toxic stage from the recovery stage. It is often desirable to study these stages separately, so it was thought advisable to determine the effect of sub-lethal concentrations of cyanide on the rate of heart beat of *Fundulus* embryos. The rate of the heart beat and the stoppage of the heart after immersion in cyanide may be taken as an index of the degree of toxicity. The resumption of the heart frequency after removal from the cyanide may give a possible indication of the rate of recovery.

METHOD AND RESULTS

Eggs were stripped from the females and fertilized in finger bowls. On the sixth day after fertilization the hearts were found to beat at a regular rate of about 136 contractions per minute. In each experiment fifty to one hundred embryos were placed in covered Stender dishes containing the cyanide solution; several of the hearts were observed and the time was taken with a stop watch for 50 cardiac contractions. It was found more accurate to determine the time for 50 contractions than to determine the number of beats per min-

ute. From time to time after immersion of the embryos in cyanide solution the beating of the auricles was observed until the heart ceased pulsating. At 30 minute intervals after the hearts stopped, ten to twenty embryos were removed from the cyanide solution, washed, and placed in normal sea water and the time of resumption of the heart beat and the rate of recovery was determined. When the hearts were immersed in cyanide, the auricles were found to beat more uniformly and were more resistant than the ventricles. Therefore, all determinations were made by observing the walls of the auricles.

The cyanide solution was made by dissolving C.P. KCN in sea water, and the following concentrations were used: M/10, M/20, M/40, M/80, and M/160. Sea water is slightly alkaline (pH 8.2) and somewhat buffered, and so no attempt was made to control the pH; however, the higher concentrations of cyanide were strongly alkaline. Brinley (1927) and Bodine (1928) showed that between pH 5 and 8 the toxicity of cyanide follows the dissociation curve, being least toxic at pH 8. As all of the experiments reported here were conducted at higher pH values than 8.2, it was thought that the H-ion concentration would not be an important factor.

THE TOXICITY OF CYANIDE AND ITS EFFECT ON THE FREQUENCY OF THE HEART RHYTHM

Six-day old embryos were immersed in KCN solutions of the following concentrations: M/10, M/20, M/40, M/80, and M/160; and the time required for cessation of the heart beat was determined. Each experiment was repeated three to five times, and the time of heart stoppage was taken when 75–80 per cent of the auricles had ceased beating. Eggs from different females showed great variation in their resistance to cyanide, but the same general types of curve were obtained in all cases. The result of a typical experiment is plotted in Figure 1, with the concentrations of cyanide as ordinates and the time required for cessation of the heart beat as abcissas. It is evident from the graph that there is a linear relation between the concentration of cyanide and the time required for cessation of the heart beat.

The effect of cyanide on the frequency of the heart beat was de-

termined at various intervals by taking the time required for 50 contractions of the auricles after the embryos were immersed in the cyanide solution. The data from a single experiment are plotted in Figure 2. During the first 15 minutes of exposure, the rate of heart beat showed a slight decrease, thereafter beating at a constant rate until just before the heart ceased pulsating. The extent of this de-

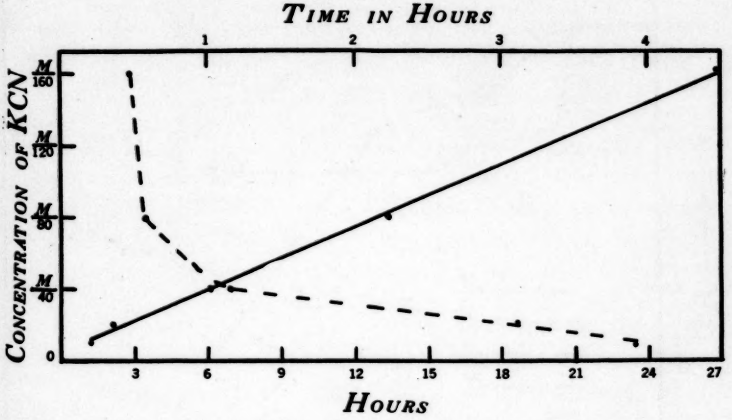

FIG. 1.—Relation of concentration to length of exposure. Ordinate represents the concentration of cyanide. The abscissa, top, represents the time in hours required for the hearts to resume beating after removal from the cyanide. Abscissa, bottom, represents the time in hours required for the heart to stop. The solid line denotes the relation of the concentration of cyanide to the exposure required for the heart to cease beating. The broken line represents the time required for the hearts to resume beating after removal from cyanide.

crease depends on the concentration of cyanide. In cases of higher concentrations of cyanide, a slight increase in the rate of cardiac contractions occurs prior to cessation of the beat; and in cases of more dilute solution, there was a gradual decrease in the rate of cardiac contractions before cessation.

RECOVERY OF THE HEART BEAT FROM THE EFFECTS OF CYANIDE

Relation of concentration to time of recovery.—After the hearts had stopped beating, the embryos were removed from the cyanide solution, washed, and placed in finger bowls of sea water, and the

time was noted when the heart resumed beating. The data from the experiments on relation of the concentration of cyanide to the time of recovery of the heart beat are expressed graphically in Figure 1. It may be seen from the chart that the curve approaches that of an inverse ratio.

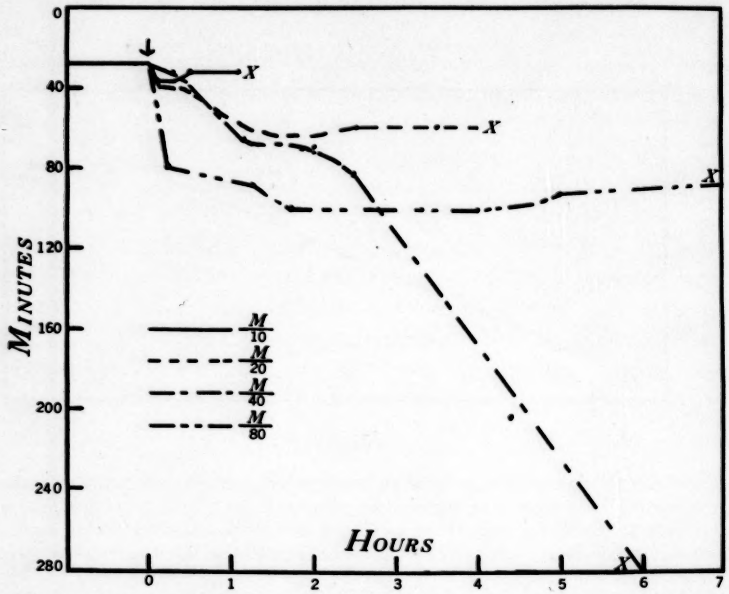

FIG. 2.—Effect of cyanide on the rate of heart beat of *Fundulus* embryos. Ordinate represents the time in minutes required for 50 cardiac contractions; the abscissa, the length of exposure in hours. The arrow indicates the time of immersion and *X* the time the heart ceased beating.

The relation of the length of exposure to recovery time.—At 30-minute intervals after the hearts stopped beating, ten to twenty embryos were removed from the cyanide solution, placed in sea water, and the time when the heart resumed was determined. The relation of the total length of exposure, and the length of exposure after the heart stopped, to the time of recovery is represented in Table I. It may be seen from the table that if the concentration remains unchanged, the time of recovery of the cardiac contractions after

removal from cyanide is constant regardless of the length of exposure. Therefore, it is evident that the recovery time depends on the concentrations but is independent of the length of exposure. This substantiates Blumenthal's (1928) conclusions on the recovery of cleavage of *Arbacia* eggs from the effects of cyanide.

TABLE I

THE RELATION OF THE CONCENTRATION OF CYANIDE TO THE TIME REQUIRED FOR THE HEART TO STOP BEATING, AND THE RELATION OF CONCENTRATION OF CYANIDE AND LENGTH OF EXPOSURE TO THE TIME REQUIRED FOR THE HEART TO RESUME BEATING AFTER REMOVAL FROM CYANIDE

Concentration of Cyanide	Time Required for Heart To Stop	Total Length of Exposure	Length of Exposure after Heart Stopped	Time Required for Heart To Resume Beating
M/10	64 min.	94 min.	30 min.	3 hr., 45 min.
		124 min.	60 min.	3 hr., 50 min.
		154 min.	90 min.	
M/20	2 hr.	2 hr., 30 min.	30 min.	3 hr., 10 min.
		3 hr.	60 min.	3 hr., 05 min.
		3 hr., 30 min.	150 min.	2 hr., 55 min.
		4 hr.	120 min.	3 hr., 15 min.
M/40	6 hr., 15 min.	6 hr., 45 min.	30 min.	1 hr., 15 min.
		7 hr., 15 min.	60 min.	1 hr., 03 min.
		7 hr., 45 min.	90 min.	1 hr., 10 min.
M/80	13 hr., 30 min.	8 hr., 15 min.	120 min.	55 min.
		14 hr.	30 min.	35 min.
		14 hr., 30 min.	60 min.	32 min.
		15 hr.	90 min.	40 min.
		16 hr., 30 min.	180 min.	38 min.
M/160	27 hr., 15 min.	28 hr., 15 min.	60 min.	27 min.
		29 hr., 15 min.	120 min.	29 min.
		30 hr., 15 min.	180 min.	26 min.

Rate of recovery.—Several embryos were removed from the cyanide solution after cessation of the cardiac contractions, and placed in normal sea water. At various intervals, after the heart had resumed beating, the time for 50 beats was taken with a stop watch. In this way the rate of recovery of the heart could be obtained. The data of a typical experiment are plotted in Figure 3. It may be noted from the graph that the rate of recovery after the heart has resumed beating is approximately constant, regardless of the concentrations of cyanide used or the length of exposure.

RECOVERY OF THE EMBRYOS IN DISTILLED WATER

Loeb and Cattell (1915) have shown that the hearts of *Fundulus* embryos poisoned with KCl would recover when returned to sea water or to solutions of certain salts but that the hearts would not resume beating when the embryos were placed in distilled water.

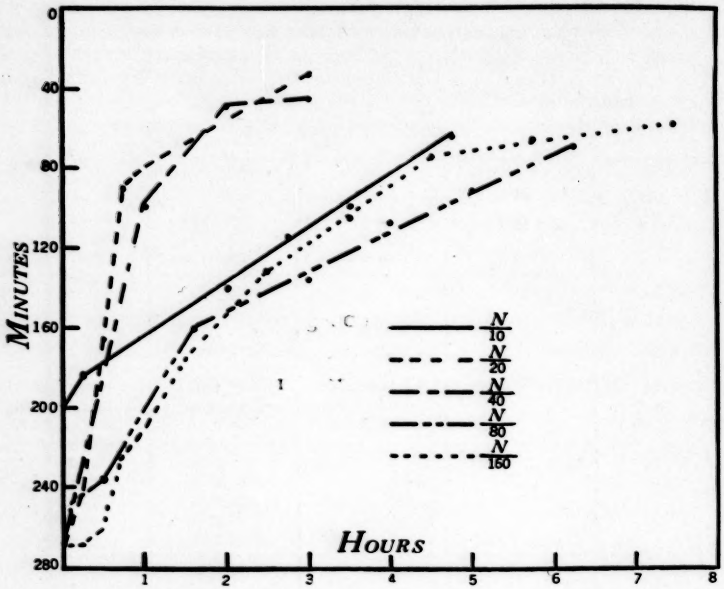

FIG. 3.—The rate of recovery of the heart beating after removal from the cyanide solution. The ordinate represents the time in minutes required for 50 cardiac contractions; the abscissa, the time in hours after the hearts resumed beating.

It was thought interesting to determine whether or not the hearts of embryos stopped by KCN would recover in distilled water. A large number of embryos were immersed in M/10 KCN until the hearts had ceased beating; then they were removed and placed in distilled water. The hearts recovered in approximately the same time as did the controls in sea water, and the embryos developed into normal fish.

CONCLUSIONS

For convenience the results may be briefly summarized as follows: (1) the toxicity of KCN to the embryonic hearts of *Fundulus* seems to be directly related to the concentration of the cyanide and to the length of exposure; (2) the recovery time of the heart beat depends on the concentration of cyanide but is independent of the length of exposure; (3) the rate of recovery of the heart rhythm is constant regardless of the concentration of cyanide or the length of exposure. These experiments present data which seem to be worthy of some further explanation. If Warburg's theory of the combination of the cyanide with the iron (haemin) in the cell is correct in general principles, we may assume that the heart stops beating before equilibrium is reached between the iron and cyanide; the reaction, however, would proceed to completion. The amount of iron combined with the cyanide at equilibrium or at any time depends on the concentration of the cyanide. The stoppage of the heart represents the formation within the cell or plasma membrane of a definite and constant amount of iron-cyanide compound. The formation of the foregoing amount of iron-cyanide depends upon the concentration and the length of exposure. Therefore, the stoppage of the heart depends upon these two factors.

The ability of the organism to recover from the toxic effects of cyanide indicate a breaking-down of the iron-cyanide compound, but not necessarily into iron and HCN. The time of recovery after immersion of the embryos in cyanide solutions depends upon the amount of iron-cyanide present within the cell or plasma membrane. In case of the higher concentrations of cyanide, a longer time is required for recovery than if more dilute solutions were used, owing to the larger amount of iron-cyanide originally present within the cell or membrane. When the amount of iron-cyanide has been reduced to a certain concentration, the heart starts beating; and the rate of recovery, which is an index to the rate of destruction of the iron-cyanide, remains constant regardless of the original concentration of cyanide or the length of exposure.

SUMMARY

Six-day-old embryos of *Fundulus heteroclitus* were placed in various concentrations of KCN solutions; the rate of heart beat and the time of cessation of the cardiac contractions was determined. At various intervals after the heart rhythm stopped, the embryos were removed from the cyanide and placed in sea water, and the time and rate of recovery was studied.

The results of the experiments seem to show that (1) the toxicity of cyanide to the embryonic hearts is directly related to the concentration and to the length of exposure; (2) the time of recovery of the heart beat depends on the concentration of cyanide but is independent of the length of exposure; (3) the rate of recovery of the heart rhythm after the heart has started to contract is constant regardless of the concentration of cyanide used or the length of exposure.

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THE EFFECT OF EXPOSURE TO COLD ON THE EMBRYONIC DEVELOPMENT OF THE JAPANESE BEETLE (POPILLIA JAPONICA NEWMAN)

(Two figures)

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INTRODUCTION

DURING the course of previous investigations, while associated with the Japanese Beetle Laboratory, United States Department of Agriculture, Moorestown, New Jersey, the writer found that eggs which had been placed, immediately after oviposition, at low temperatures (approximately 10° C.) for one month were retarded in their subsequent development at constant high temperatures. Bodine (1925 and 1929) and Parker (1927) found that low temperatures under normal environmental and experimental conditions produced a very marked acceleration in the development of grasshopper eggs. Since previous results with the Japanese beetle showed a retardation rather than an acceleration, as a result of exposure in low temperatures, it was decided to repeat these investigations and also to study, first, the extent of retardation when the eggs were exposed to cold for different periods of time and, second, the effect of exposure during different embryonic stages on the rate of subsequent development.

MATERIAL AND METHODS

Nine hundred and fifty eggs were used in these experiments. They were collected daily from beetles kept in the laboratory and placed in metal salve boxes containing moist soil. The boxes and the soil had been previously sterilized for ½ hour in a hot-air sterilizer at 150°–200° C. They were then divided into series (each of which contained from twenty to forty eggs), and a series was placed under each of the following experimental conditions: 8°–12° for 1 week, then at 30° C.; 30° for 1 day, 8°–12° for 1 week, then at 30° C.; 30° for 2 days, 8°–12° for 1 week, then at 30° C.; etc. In this manner

eggs were permitted to develop from 1 to 7 days before exposure to cold for 1 week, after which they were kept at 30° C. Parallel series were allowed to develop from 1 to 7 days at 30°, exposed to cold for 2 weeks, then placed at 30°; other series were placed at 30° from 1 to 7 days, exposed to cold for 3 weeks, then placed at 30°; while others were placed at 30° from 1 to 7 days, exposed to cold for 4 weeks, then placed at 30°.

In calculating the time required for embryonic development, only those days during which the eggs were at 30° C. were counted, since temperatures of 8°–12° C. are presumably below the threshold for the development of the Japanese beetle (Ludwig, 1928). Thirty degrees (constant to within one-half degree) was selected as the high temperature because, in the work just cited, it was found to be the optimum for embryonic development of this species. The humidity was kept approximately constant (near the point of saturation) by the addition of tap water to the soil.

Many of the eggs which had been exposed to cold for 3 or 4 weeks developed into larvae which were weakened to such an extent that they were unable to free themselves completely from the egg shell. Consequently, the time of hatching was taken when the larva had succeeded in rupturing the egg shell with its mandibles.

RESULTS

Effect of exposure to cold for varying periods of time.—A retardation in the rate of embryonic development was produced in each series. The degree of retardation seemed to depend on the length of time the eggs were exposed to cold. Ludwig (1928) found that at a constant temperature of 30° C. the rate of development of the eggs was very uniform, the minimum time required being 7 days, the maximum 8 days, and the average 7.98 ± 0.018 days. When eggs were exposed to cold for 1 week, the minimum was 7 days, the maximum 16 days, and the average 8.5 ± 0.047 days; 2 weeks' exposure gave a variation of from 7 to 19 days, with an average of 9.4 ± 0.183 days; while 3 weeks' exposure produced a range of from 8 to 23 days, with an average of 11.8 ± 0.34 days. The results of exposure for 4 weeks were not significantly different from those obtained from eggs exposed for 3 weeks. These results are shown graphically in

Figure 1. The percentage of eggs which hatched under the various experimental conditions is also plotted in this figure. The percentage curve is practically the reciprocal of the time curve. This probably means that exposure to cold produces not only retardation of development but also a greater mortality among the developing larvae.

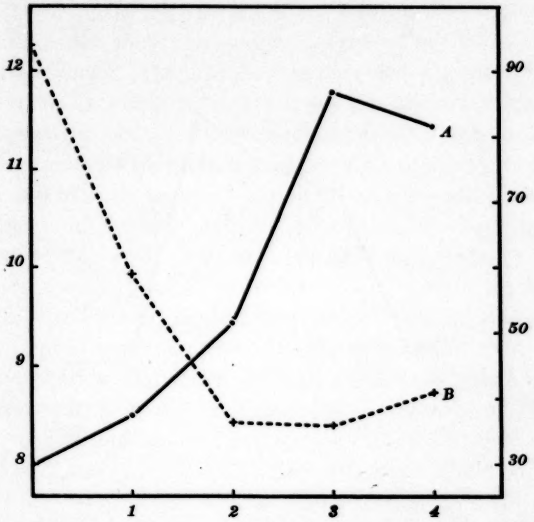

FIG. 1.—Curves show effect of exposure to low temperatures (8°–12° C.) for varying periods on length of embryonic stage. Abscissas, time exposed to cold in weeks; left ordinates, days required for embryonic development; right ordinates, percentage of eggs which hatched. *A*, time curve; *B*, percentage curve.

Effect of exposure to cold during different embryonic stages.—Figure 2 shows that exposure to cold produces retardation during all stages of embryonic development. However, the extent of retardation is greater in eggs which were allowed to develop for 3, 4, or 5 days before being placed in the cold than in those exposed near the beginning or end of embryonic development. In Figure 2 the percentage curve is also nearly the reciprocal of the time curve. The only exception is found in eggs placed in the cold as soon as they were collected. In these series retardation is least, the time required for hatching being only 8.8 ± 0.168 days; however, only 7.7 per cent of

the eggs hatched. When the egg of the Japanese beetle develops normally, it absorbs water very rapidly and increases to several times its original size within 1 or 2 days. Very few of the eggs exposed to cold immediately after oviposition showed such signs of development.

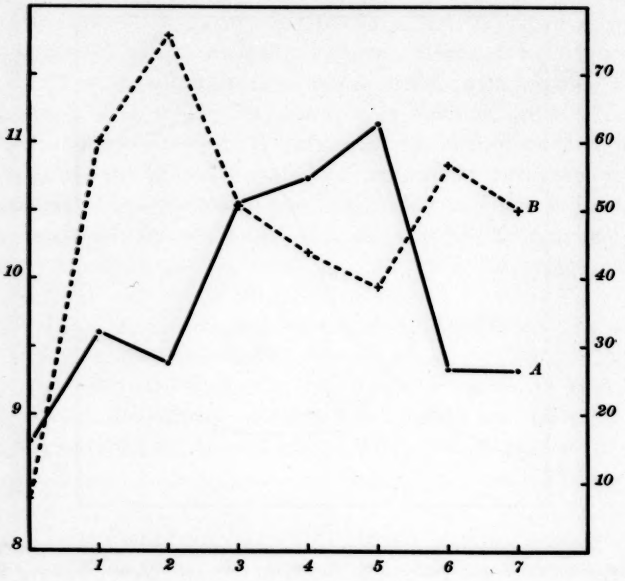

FIG. 2.—Curves show effect of exposure to low temperatures (8°–12° C.) at different stages of embryonic development. Abscissas, days kept at 30° C. before exposure to cold; left ordinates, days required for embryonic development; right ordinates, percentage of eggs which hatched. *A*, time curve; *B*, percentage curve.

DISCUSSION

Under normal field conditions, the eggs of the Japanese beetle are laid during the months of July and August and hatch before the advent of cold weather. The winter is always passed in the larval stage. Hence, normally, the eggs are probably not exposed to temperatures below the threshold for development. They are therefore comparable to grasshopper eggs which normally are never exposed to low temperatures and which ordinarily do not hatch if thus

treated (Class II in Bodine's classification of grasshopper eggs [Bodine, 1925]). These experiments of Bodine and those of Parker (1927), in which they obtained an acceleration of development after exposure to low temperatures, were performed on grasshopper eggs which are normally frozen (Class I). That these eggs are physiologically very different from those of Class II is shown by Bodine (1929). He found that eggs which normally are subjected to cold show cyclic or rhythmic changes in the rates of oxygen consumption and of development which are apparently independent of environmental conditions, while eggs which are usually never subjected to low temperatures do not show these rhythmic changes. The fact that, in the present experiment, exposure to cold produced a retardation rather than an acceleration of development is evidence of physiological differences between Japanese-beetle eggs and grasshopper eggs of Class I. This may possibly be correlated with seasonal life-history.

Bodine (1925) found rhythms in the susceptibility of the grasshopper egg to low temperature. If eggs were exposed to 10° C. immediately after laying, there was no acceleration when placed at high temperatures. However, if the eggs of *Melanoplus differentialis* were allowed to develop at least 2 weeks at 20° C., they entered a period of high susceptibility; this, in turn, was followed by a period during which exposure to cold produced no change in rate of development. Loeb (1915), Kellicott (1916), and Stockard (1921) reported rhythmic changes, in the susceptibility to low temperature, in the developing *Fundulus* egg. There are stages of apparent indifference which are followed by stages of extreme sensitiveness.

The results obtained in the present study of the Japanese-beetle egg also show rhythmic changes in susceptibility to cold. First, there is a period during which exposure produces a very high mortality but very little retardation in the rate of development; second, there is a period of decreased susceptibility as measured by mortality but of increased susceptibility as measured by the retardation produced; third, there is a period of still greater susceptibility during which both the mortality and retardation of development are increased; and fourth, there is a period near the end of embryonic development during which both the mortality and extent of retarda-

tion are again decreased. It is planned to correlate, if possible, these changes in susceptibility with morphological changes in the egg during development.

SUMMARY

1. Exposing the eggs of the Japanese beetle to low temperatures results in a retardation in the subsequent development of the eggs at a constant high temperature.

2. The results indicate rhythmic changes in susceptibility to low temperatures during embryonic development.

The writer is indebted to Doctor Henry Fox, of the Japanese Beetle Laboratory, United States Department of Agriculture, Moorestown, New Jersey, for some of the material used in these experiments.

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ASEXUAL REPRODUCTION IN NEMERTEANS

(One figure)

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IN MANY species of nemerteans, or ribbon worms, the body is so slender and fragile that it is difficult to secure an unmutilated individual. And even after such a specimen is obtained, it is liable to fragment spontaneously in any unnatural environment or under any extraordinary stimulus.

Not every species of the group is subject to such behavior, however, and as a rule the more slender the body the greater the liability of fragmentation. It might be expected that the more fragile species would have evolved a compensatory capacity for regeneration, but this is not usually the case. For in the vast majority of species, including even the slender forms, only the anterior portion of the body, provided with the head, is capable of survival and regeneration; all the other fragments disintegrate within a few days or a few weeks. There are certain notable exceptions, however, for some species are known which possess a regenerative capacity equaled by representatives of few other groups of invertebrates. These highly regenerative species all belong to the genus *Lineus*. They are represented on the coasts of Europe and on both the eastern and western coasts of the United States (Nusbaum and Oxner, 1910, 1911, 1912; Monastero, 1928; McIntosh, 1870; Brown, 1897; Coe, 1929).

In those species in which the regenerative ability is developed to the highest degree, a minute fragment cut from almost any portion of the body soon restores all the missing organs and reorganizes itself into a miniature of the original worm. Even a sector of one of these minute pieces, if it contains a piece of the lateral nerve, is likewise able to form the entire body. Hundreds of simultaneously regenerating individuals can be obtained from a single worm (Coe, 1929).

The question arises as to whether fragmentation, with the subsequent regeneration of the severed parts, is a normal process of

agamic reproduction in any of these highly regenerative forms. McIntosh (1870) considered such reproduction as merely facultative, but Brown (1897) assumed a close correlation between regenerative capacity and asexual reproduction by fragmentation in *Lineus gesserensis*. Nusbaum and Oxner (1910), on the other hand, ridiculed Brown's idea and insisted that fragmentation never occurs normally in *L. ruber*, which they considered specifically identical with *L. gesserensis*. They assert that whenever fragmentation occurs in *L. ruber* it is the result of infection and that regeneration of the fragments does not occur. This is my own experience with *L. ruber*, which also occurs abundantly on our Atlantic Coast from Long Island Sound northward. There are many intergrading color varieties of the latter species, the green variety frequently being called *L. viridis* (Verrill). Often associated with *L. ruber* in the Woods Hole region is a distinct species, *L. socialis* Leidy. The two species bear a close superficial resemblance but differ in the relative thickness of the muscular layers, in position of ocelli, and in other morphological details. They are readily distinguished when fully contracted, for *L. ruber* then becomes short and thick while *L. socialis* coils the body into a close spiral. They also differ profoundly in regenerative capacity, for *L. ruber* is capable only of posterior regeneration if cut posterior to the head, while an individual of *L. socialis* may be cut into a hundred or more pieces, every one of which will regenerate into a minute replica of the original.

A similar distinction presumably holds between the species (*gesserensis*) reported by Brown (1897) and that referred to as *ruber* by Nusbaum and Oxner (1910). The latter investigators were doubtless in error in assuming the specific identity of the two forms, although they experimented with both and called attention not only to the difference in the regenerative capacity of the two but also to well-marked morphological distinctions. Hence their criticism of Brown's assertion that asexual reproduction occurs in *L. gesserensis* seems quite unjustified.

Certainly as regards *L. socialis* there can be no reasonable doubt that agamic reproduction by fragmentation and subsequent regeneration of the fragments is an important factor in the propagation of the species. A closely related species (*L. vegetus*) on the

Pacific Coast has a similar behavior. A mature individual of either of these species, left undisturbed for a few days in clean sea water is found to have broken off from the posterior half of its body a number of fragments of various sizes. Each fragment will quickly regenerate into a small replica of the original, so that after a few weeks the single individual will have been replaced by perhaps six to twenty or more worms of smaller size (Fig. 1). These small

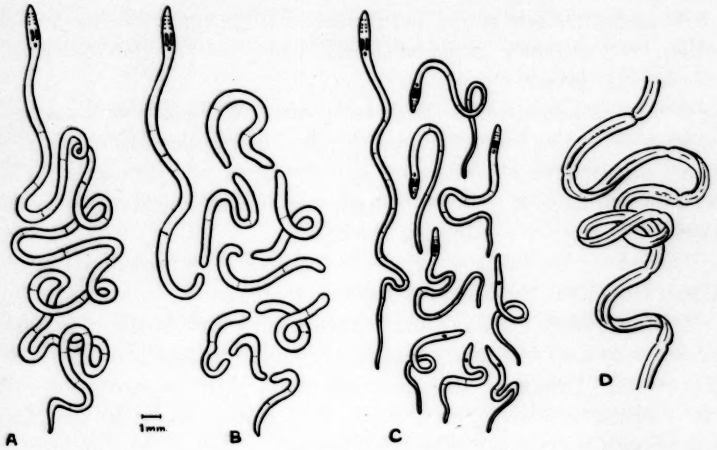

FIG. 1.—Diagram of agamic reproduction in *Lineus socialis*. *A*, mature worm; *B*, same after producing 8 fragments; *C*, regeneration of each fragment, resulting in 9 smaller worms of normal proportions; *D*, portion of a body of mature worm, showing zones of fission.

worms are quite indistinguishable from the small individuals found under stones along the seashore, and will remain alive in the laboratory for several months without food.

In order to determine the conditions and frequency of such spontaneous fragmentation in *L. socialis*, thirty worms ranging in size from about 30 to 90 mm. in length were selected at random and isolated in separate jars of sea water. The water was changed daily during the first 10 days, care being taken not to disturb the worms unnecessarily. At all other times they were left in the dark at a temperature approximating 18°–22° C.

No fragmentation took place during the first day, but at the end of the second day six of the thirty worms had broken off the posterior portions of their bodies. One had produced 6 fragments in addition to the anterior portion, two had formed 3 posterior fragments each, one had 2, and two had each cut off a single fragment.

On the third day thirteen worms had fragmented, with an average of about 3 pieces each; and on the fourth day all but five had formed fragments.

After 7 days one worm had formed 9 fragments, another 8, two others 7 each, three had 6 each, and only two remained with the whole body intact.

At the end of 2 weeks the anterior portion of one worm was accompanied by 11 fragments, another had 9, another 8 (Fig. 1), and three others 7 each, while two still remained unfragmented. The average number for the whole group was about 4 fragments in addition to the portion containing the head.

During the following week two individuals each formed one additional fragment; the others showed no increase.

Ten days later (31 days after capture) five individuals were each accompanied by one additional piece, while one had formed 2 new fragments. Two were still unfragmented. Without exception, all the fragments were in process of rapid regeneration, so that the conditions for existence were not obviously unfavorable. The water remained so free from excretion products that it was changed only once each week after the first 10 days.

At the end of 2 weeks ten of the thirty worms were used for other experiments, and the remaining twenty were continued under observation. Even after more than a month in confinement ten of these twenty continued to form new fragments. From the thirty-first to the forty-first day one worm added 4 pieces to those previously produced, two others added 3 fragments each, one formed 2, while each of three others produced a single additional piece. During the following 10 days one of the longest worms, which had previously formed only 2 fragments, added 3 more, and three others added 1 fragment each. Thus, on the fifty-first day a total of 100 fragments had been produced by eighteen of these individuals, while two still remained with the original body intact. The average for the entire

group is therefore exactly 5 fragments each in addition to the piece bearing the original head; that is, the original twenty worms are now represented by 120 regenerated individuals of smaller size.

By the sixty-eighth day, one of the two worms previously unfragmented had produced a single new piece, one with 9 pieces had added 3 more, and one with 6 had an increase of 1, making a total of 125 regenerating individuals, in place of the original 20. The single worm which remained unfragmented was originally the smallest of the lot and not improbably represented an individual which had been previously derived from a fragmented piece.

TABLE I

Original Length (Mm.)	No. of Worms	No. of Fragments from Each	No. of Regenerated Individuals
30	1	0	(1)
35	1	12	13
40	4	1, 3, 4, 7	2, 4, 5, 8
45	1	2	3
50	1	4	5
55	1	5	6
60	3	1, 2, 4	2, 3, 5
70	3	4, 6, 8	5, 7, 9
80	4	5, 6, 8, 11	6, 7, 9, 12
90	1	12	13
Total	20	105	125

At the end of 3 months there had been practically no further increase in number. Two of the worms had added 1 fragment each, the unfragmented small individual and one of the others were dead, and most of the small regenerated worms had become pale in color and decreased in size.

The number of fragments produced is correlated somewhat closely with the length of the worm, as is shown by Table I, which indicates the original length of each of twenty worms and the number of fragments which it had formed at the end of 68 days in captivity. The table also shows that without exception every fragment regenerated into a smaller replica of the original worms.

The table shows that the shortest worm (30 mm.) formed no fragments, while the longest (90 mm.) formed 12. The next shortest (35 mm.) formed as many fragments (12) as the longest, and one

having a length of only 40 mm. formed 7. With these two notable exceptions none of the individuals under 70 mm. in length formed more than 5 fragments, and only four of those 70 mm. or over formed less than 8.

For another experiment, seventy-four worms were collected near New Haven, Connecticut, August 15, 1929. These were kept in the dark at a nearly uniform temperature of about 16°. The temperature was from 2° to 6° cooler than in the preceding experiment on worms collected at Woods Hole, Massachusetts, and 6°–10° cooler than the seashore at that season of the year.

The rate of fragmentation was correspondingly reduced; only 57 per cent of the worms had formed fragments at the end of 7 days, while in the earlier experiment 90 per cent had fragmented within a similar period.

By the twelfth day fifty-six of the seventy-four had produced a total of 247 fragments (not counting the original head-pieces), the percentage of fragmentation at this time being about 74.

During the following 7 days seven more individuals fragmented, bringing the percentage to 85. The total number of fragments was 386, an average of slightly more than 6 fragments for each of the sixty-three participating individuals.

At the end of 26 days only nine of the seventy-four worms were still unaccompanied by regenerating fragments, the total number of the latter being 430. Eleven days later (after 37 days in captivity) there were still nine unfragmented individuals, but the number of regenerating pieces had been increased to 531. The largest worm of the original collection had produced 23 regenerating individuals, the average number for the sixty-five fragmented worms being 8.

Worms brought directly from the seashore and kept at temperatures of from 10° to 5° or less remain almost inactive and show very little tendency to fragment. Moreover, such fragments as are formed have a decreased regenerative capacity at these low temperatures. Consequently this method of reproduction is normally interrupted during the winter months, thereby giving the matured regenerated individuals an opportunity to form their sexual products.

Numerous experiments indicated not only that the rate of fragmentation is retarded by a decrease in temperature but that the

rate is not greatly affected by the presence or absence of illumination by indirect sunlight. But as a further test as to the effect of various environmental conditions, eighty-two worms, collected near New Haven, Connecticut, September 28, 1929, were kept in a room lighted by indirect sunlight and at a temperature approximating 21°. The water was changed only when visible waste materials had accumulated. Some of the bottles, each containing a single worm, were provided with sand, gravel, and broken shells taken from the worm's natural habitat. To others a small quantity of mud was added.

Within 4 days forty of these eighty-two worms had formed 84 fragments; and within 8 days seventy-three, or nearly 90 per cent, had fragmented, producing a total of 312 regenerating individuals in addition to the original number. Only five of the worms which remained intact after 8 days exceeded 50 mm. in length.

The results of this experiment agree almost exactly with those of the Woods Hole lot kept at a similar temperature, but in the dark, indicating that an increased temperature is a much more potent factor in accelerating the rate of fragmentation than is the presence or absence of light.

The presence of gravel, mud, and other materials was without appreciable effect, for fragmentation took place with about the same frequency as when the bottles contained only sea water. The worms showed the same tendency to crawl up on the sides of the glass, and often above the surface of the water, in spite of the opportunity provided them for remaining among the materials taken from their normal habitat.[1]

The retarding influence of a lower temperature on the rate of fragmentation is illustrated by six worms, varying in length from 60 to 120 mm., which were kept at a temperature of 10°. After 26 days all remained unfragmented except one, which had produced a single fragment. A worm 150 mm. long, after having fragmented

[1] It should be noted in connection with this habit that the natural habitat of the species is above the middle of the intertidal zone along the seashore, where the stones beneath which the worms live are exposed to the air for several hours at each low tide. In common with so many other invertebrates living in similar situations, this species often responds to the changed environment by creeping above the surface of the water occasionally.

once, was also kept 26 days at the temperature mentioned without forming any additional fragments. When returned to 21°, however, fragmentation took place within a few days.

As the water along the seashore becomes colder in autumn, fragmentation is doubtless similarly inhibited, the worms increase in length, and their sexual products develop in anticipation of the period of sexual reproduction which occurs in the winter.

By the middle of November the water in Long Island Sound has already reached the inhibiting point of 10° or under, and collections of worms made at this time show few cases of fragmentation unless the temperature is raised. Regeneration is also less complete when the body is filled with sexual products than at other seasons of the year, as Van Cleave (1929) has recently shown for *Stenostomum*.

Of twenty-four worms collected November 16, 1929, and kept at approximately the same temperature (9°) as the water along the seashore at that season, none had fragmented at the end of 7 days. After 22 days only three had broken off fragments; one of the three was obviously abnormal and had extruded its proboscis, the second had produced 4 fragments, and the third only 1—a condition in marked contrast with the rapid asexual reproduction occurring in the summer months.

These results are in complete harmony with my observations on this species during many past years, and at different seasons of the year, although no records were kept previously as to the actual number of fragments produced by each worm under various conditions.

While it cannot be proved that this method of agamic reproduction takes place regularly in the life-cycle under a natural environment, it may be assumed that it is at least of frequent occurrence. In the laboratory experiments the only obviously abnormal conditions are absence of tidal changes and particularly the absence of food. Frequent changes of the water prevent an accumulation of excretory products and assure an abundant supply of oxygen. Certainly the conditions of the experiments are such as to allow the rapid and normal regeneration of every one of the numerous fragments produced.

Careful examination of the worm's body, both in this species and

in *L. vegetus*, shows that provision for fragmentation is anatomical as well as physiological. Crossing the body at intervals is a series of narrow transverse lines, or rings, of lighter color because devoid of the pigment which occurs elsewhere on the body (Fig. 1). There are commonly four or five such cross-lines in the esophageal region and seven to twelve in the intestinal region, but occasionally the number in the esophageal region is increased to seven or eight, with twenty or more on the rest of the body.

Brown (1897) mentions these delicate transverse lines in *L. gesserensis* and states that the zones of fission coincide with them exactly. His statements concerning the mechanics of the fission process, however, seem to be somewhat erroneous, for he explains it as resulting from a solution or digestion of the body walls through the liberation of digestive fluids from the alimentary canal at the zone of cleavage. In *L. socialis* I have found no evidence to support this view, the spasmodic local contraction of the circular and longitudinal musculatures of the body walls being sufficient to cause the rupture of all the tissues in the zone of fission. These contractions, however, are repeated many times before separation of the parts actually occurs. Deep constrictions appear and disappear on the body over and over again, until one or more of them eventually severs the body completely.

Such constrictions frequently, but not always, coincide with the narrow transverse lines mentioned above, but they rarely occur simultaneously in all parts of the body. Undoubtedly the lines indicate zones of weakness in the body walls, as has recently been found to be the case with similar rings on the body in certain species of annelids (Allen, 1927).

In spontaneous fragmentation, the initial rupture may take place at any point in the posterior two-thirds of the body. Consequently the first fragment may be very short or it may be twice as long as the anterior portion containing the head, but it is usually less than half the length of the original body. Succeeding fragments may be cut off either from the posterior end of the anterior piece or from either end of the fragment first formed. The process may be repeated until only a small part of the intestinal region remains attached to the anterior piece and until all the rest of the body is cut

into small segments. Occasionally even the esophageal region is involved. All fragments are equally capable of regeneration.

The tendency to fragment is by no means limited to the mature worms, for small sections cut from the body, particularly if they be somewhat irregular in shape, frequently break up into several pieces during the progress of regeneration. Each of these pieces likewise regenerates, so that several minute individuals may develop from a single section (Coe, 1929). Also, when two pieces, either from different parts of the same worm or from different worms, are grafted together, the partially regenerated organism sometimes splits into two or more separate individuals.

It seems not improbable that this method of asexual reproduction may be of frequent occurrence during the lifetime of the worm. Possibly only one or two fragments are usually formed at intervals instead of the more numerous pieces produced in these experiments, and presumably accidents or abnormal conditions of various kinds lead to fissions which would otherwise not have occurred. The worm may thus produce many offspring before reaching sexual maturity, and there seems to be no reason for assuming that the process may not be repeated over and over again in the regenerated individuals as soon as they have grown to a reasonable size. Sexual reproduction in some family lines may thereby be deferred indefinitely.

In the laboratory no growth has taken place in the regenerated individuals because no means of feeding them has been devised. On the contrary, they become gradually smaller, and most of them die after a starvation period of 3 to 8 months. Occasionally one of the starving worms encysts and goes through a process of involution to very simple tissues.

Not only does this capacity for asexual reproduction allow for a great increase in the number of individuals derived from a single mature worm, but all these new individuals are presumably young physiologically, as is known to be the case with planarians of similar origin. Hence it is not inconceivable that occasions may arise in nature when this ability may be an important factor in the survival of local communities.

SUMMARY

1. In two species of ribbon worms spontaneous fragmentation, with regeneration of each fragment, is considered a normal method of agamic reproduction, alternating with an annual sexual phase.

2. By this process from two to more than twenty smaller individuals of normal proportions and presumably young physiologically may be produced by each fragmenting worm.

3. In the summer months nearly 90 per cent of the mature worms kept under conditions as nearly normal as possible reproduce in this manner within 7 days after being brought from the seashore.

4. During a period of 68 days 125 smaller, regenerated individuals resulted from twenty worms kept under favorable conditions. Another lot of eighty-two worms produced 394 regenerating fragments within 8 days, while a third lot of seventy-four worms was represented by 531 small ones after 37 days.

5. Such asexual reproduction normally occurs during the warmer months of the year, being inhibited by low temperature in the autumn, at which time the sexual products to be discharged during the winter are beginning to form.

6. By raising the temperature of the water above 16°, fragmentation can be brought about even after the sexual products are nearly mature, but regeneration at this time is less certain and frequently long delayed.

7. The longer worms produce, as a rule, more fragments than do smaller individuals; at least some of the latter may have been recently derived from the regeneration of fragments previously produced by other worms.

8. It seems not improbable that under certain conditions the process may be repeated many times or perhaps indefinitely, with a corresponding postponement of sexual reproduction.

9. Correlated with autotomy is an amazing capacity for regeneration when the body is cut into minute pieces.

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A STUDY OF THE MOTILITY AND RESISTANCE OF RAT SPERMATOZOA AT DIFFERENT LEVELS IN THE REPRODUCTIVE TRACT[1]

(One figure)

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INTRODUCTION

IN A former paper (Yochem, 1929) the writer has studied the viability of mammalian spermatozoa after their introduction into the female reproductive tract by normal and artificial methods. Having determined that their vitality was retained for but a few hours within the female tract, my interest was turned to a study of these highly specialized cells within the male reproductive tract. Such problems as the reaction of the spermatozoa to different temperatures, degrees of alkalinity and acidity, and their normal motility at different levels of the reproductive tract have all been of interest. These problems have been approached by a study of the sperm *in vivo* dealing with differences in motility, and *in vitro* by removing sperm from different levels of the reproductive tract and subjecting them to various temperatures, alkalinity and acidity, and saline solutions.

I wish to express my indebtedness to Dr. L. B. Arey for numerous suggestions and for counsel and guidance during the progress of this problem.

PROCEDURES AND OBSERVATIONS

The data to be given include a study on approximately 95 male white rats, and it is believed that sufficient observations were conducted to minimize the occasional variable results encountered in this type of problem.

The spermatozoa were removed from the reproductive tract as follows: After killing the animal by a blow on the head, the abdomen was sufficiently opened to expose the testis, epididymis, and

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ductus deferens. Following this, the desired level (cf. Fig. 1) was extirpated with small scissors, care being taken to prevent contamination of one part with sperm from another. The portions thus obtained were transferred to small vials containing from about ½ to 1 cc. of the solution in which the spermatozoa were to be studied. The sperm were released from each part by hashing finely with small scissors, after which the excess tissue was removed with forceps. Several drops of this sperm suspension were placed in a depression slide and examined microscopically for spermatozoan activity. The low power of the microscope was sufficient to detect the slightest movement on the part of even a single spermatozoön. Each step described was conducted as quickly as possible in order to eliminate possible errors due to loss of motility.

To estimate the rate of motility of spermatozoa in rough quantitative terms, the method adopted by Moore has been used. Thus, four asterisks (****) are used to designate the highest vitality of sperm movement; in such cases all visible spermatozoa are in vigorous movement. When few sperm are immotile and the entire mass less vigorous in their movement, three asterisks (***) have been used; likewise, two asterisks (**) and one asterisk (*) indicate progressively weaker vitality; and the entire absence of motility is designated by o.

I. THE MOTILITY AND RESISTANCE OF SPERMATOZOA AT DIFFERENT LEVELS OF THE REPRODUCTIVE TRACT IN VIVO

In order to determine what effects abdominal temperature may have on spermatozoa at various levels, the desired parts of the tract were isolated with ligatures and confined within the abdomen.

The animal was anesthetized with ether, and the abdomen was shaved and sterilized. Through a low abdominal incision the testis, epididymis, and ductus deferens[1] were carefully retracted through the opening of the inguinal canal and brought into the field of operation. Subsequently, the remnant of the gubernaculum, which is attached to the tail of the epididymis and bottom of the scrotal sac, was cut. Both testes were removed, thus eliminating the supply of spermatozoa. All vessels and nerves were left intact, and the parts

[1] It should be remembered that the greater portion of the ductus deferens is normally within the scrotal sac.

of both sides were confined within the abdomen by closing the inguinal opening with a purse-string suture. Ligatures were placed between the head and tail of the epididymis, and the proximal and distal ends of the ductus deferens of the right side (cf. Fig. 1), care being taken to avoid all blood-vessels. Such ligatures prevented possible migration of the spermatozoa down the tract. The opposite (left) side was undisturbed after it was withdrawn into the abdomen and was used as a control. But it should be remembered that some sperm on the unligated side would perhaps pass into the ductus deferens. Following the foregoing procedures, the abdomen was closed with silk thread and recovery allowed.

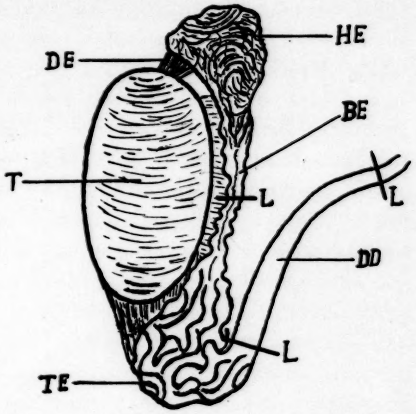

FIG. 1.—Testis, epididymis, and ductus deferens of the rat, illustrating the position of ligatures used in isolating segments of the seminal duct for the experiments described in the text. *HE*, *BE*, *TE*, head, body, and tail of epididymis; *DD*, ductus deferens; *DE*, ductuli efferentes, which are cut when testis is removed; *L*, positions of ligatures.

The animals were sacrificed from 3 to 8 days after the operation, and the isolated regions examined for motile spermatozoa. The sperm were removed as already described and placed in normal physiological saline solution (0.9 per cent) in order to study their motility.

Table I presents the data of observations on 19 animals. Three days after confinement in the abdomen there is a slight loss of motility in the spermatozoa taken from the head and tail of the epididymis and ductus deferens. After 4 days there is a relatively greater loss of motility in the spermatozoa taken from the head of the epididymis than in those recovered from the tail of the epididymis or ductus deferens. Five days after confinement in the abdomen almost the same conditions are present as after 4 days, except that the movement of the spermatozoa in the ductus deferens begins

to decrease; similar observations were noted 6 days post-operation. No live sperm were recovered from the head of the epididymis or ductus deferens after 7 days; only a few feebly motile spermatozoa were found in the tail of the epididymis, and these in but a few cases. Four animals were examined 8 days after operation. Only a few

TABLE I

BILATERAL CASTRATION: CONFINEMENT OF THE EPIDIDYMIS AND DUCTUS DEFERENS FROM EACH SIDE IN ABDOMEN

Days after Operation	Right Side†			Left Side		
	Head of Epididymis	Tail of Epididymis	Ductus Deferens	Head of Epididymis	Tail of Epididymis	Ductus Deferens
3	**	***	***	**	***	**
3	**	***	**	*	***	**
4	o	***	***	o	***	***
4	o	**	*	*	**	*
4	*	**	**	o	**	**
5	o	o	o	*	**	**
5	o	**	**	o	**	**
5	o	**	*	*	**	**
5	*	**	*	o	**	**
5	**	*	o	o	*	*
6	o	o	o	o	*	*
6	*	**	*	*	*	*
7	o	*	*	o	*	*
7	o	*	o	o	o	o
7	o	o	o	o	o	o
8	o	*	o	o	*	*
8	o	o	o	o	o	o
8	o	o	o	o	o	o
8	o	o	o	o	o	o

† Ligatures on right side only.

NOTE.—The degree of motility in an observed field has been rated to the following arbitrary symbols: Thus, four asterisks (****) are used to designate the highest vitality of sperm movement; in such cases all visible spermatozoa are in vigorous movement. When few sperm are immotile and the entire mass less vigorous in their movement, three asterisks (***) have been used; likewise, two asterisks (**) and one asterisk (*) indicate progressively weaker vitality; and the entire absence of motility is designated by o.

Normal motility for the several parts are: head of epididymis ***, tail of epididymis ****, ductus deferens ****.

slightly motile spermatozoa were recovered from the tail of the epididymis of one animal. A few spermatozoa with very slight movement were observed in the ductus deferens of the left side in one animal, but perhaps these had migrated from the tail of the epididymis since there were no ligatures on that side.

The tabulated observations clearly show that the spermatozoa at different levels of the genital tract are not equally affected by confinement in the abdomen. The relative progressive loss of mo-

tility is more rapid in the head of the epididymis than in any other part. The sperm in the ductus deferens are killed a little later, and finally those in the tail of the epididymis are the last ones to lose motility.

No morphologic changes were observed in dead or live spermatozoa. However, the epididymides appeared somewhat glassy or opaque in a few cases of prolonged abdominal confinement, whereas normally the organ is dull white or milky in appearance. Furthermore, slight adhesions of the tract were observed in some cases, and two animals were not examined because of total disorganization of the parts ligated.

The observations just described seem to indicate that abdominal temperature inactivates the spermatozoa in the head of the epididymis first, then those in the ductus deferens, and finally those in the tail of the epididymis. However, it was necessary to duplicate the foregoing experiment and allow the genital tract to remain in its normal environment (the scrotal sac) before concluding that abdominal temperature affects spermatozoa differently at different levels.

The operations and procedures in this experiment were conducted as already described, with the exception that ligatures were placed on both sides, the right epididymis and ductus deferens were confined in the abdomen, and the left tract was replaced in the scrotum.

Moore (1928) observed motile rat spermatozoa in the epididymis for approximately 14 days after the testis was removed, if the epididymis was replaced in the scrotum. And Heller (1929) showed that this period for persistence of motility was reduced to 6 or 7 days when the testis was removed and the epididymis was confined in the abdomen. In their experiments the whole epididymis was examined for motile spermatozoa, rather than any specific level. Table II shows a record of the findings in 22 animals. It should be remembered that the right epididymis and ductus deferens were abdominally confined, while the left were replaced in the scrotal sac.

The animals were killed at increasing time-intervals from 7 to 16 days, and the regions were examined for motile spermatozoa as already described.

The observations on the right (abdominally confined) epididymis and ductus deferens require little explanation. A few feebly motile spermatozoa were recovered from the tail of the epididymis of one animal; only dead sperm were found in all the other levels of both animals 7 days post-operation. One case extended motility in the

TABLE II

BILATERAL CASTRATION: RIGHT EPIDIDYMIS AND DUCTUS DEFERENS IN ABDOMEN; LEFT EPIDIDYMIS AND DUCTUS DEFERENS IN SCROTUM

Days after Operation	Right Side			Left Side		
	Head of Epididymis	Tail of Epididymis	Ductus Deferens	Head of Epididymis	Tail of Epididymis	Ductus Deferens
7	o	*	o	**	**	**
7	o	o	o	*	o	o
8	o	o	o	**	**	**
8	o	*	o	*	***	**
10	o	o	o	o	**	**
10	o	o	o	*	**	**
10	o	o	o	o	*	o
10	o	o	o	o	o	o
10	o	o	o	o	*	*
10	o	o	o	o	*	*
10	o	o	o	o	*	o
10	o	o	o	o	**	**
12	o	o	o	o	*	*
12	o	o	o	o	o	o
14	o	o	o	o	*	o
14	o	o	o	o	o	o
16	Not examined after 14 days			o	*	o
16				o	o	o
16				o	o	o
16				o	o	o
16				o	o	o
16				o	o	o

NOTE.—The degree of motility in an observed field has been rated according to the following arbitrary symbols: Thus, four asterisks (****) are used to designate the highest vitality of sperm movement; in such cases all visible spermatozoa are in vigorous movement. When few sperm are immotile and the entire mass less vigorous in their movement, three asterisks (***) have been used; likewise, two asterisks (**) and one asterisk (*) indicate progressively weaker vitality; and the entire absence of motility is designated by o.

Normal motility for the several parts are: head of epididymis ***, tail of epididymis ****, ductus deferens ****.

tail of the epididymis to the eighth day, and then only a few spermatozoa showed a weak vibratile movement. Eight animals were examined 10 days after confinement of the tract in the abdomen, and in no case were live spermatozoa recovered from any level. Four animals examined after 12 to 14 days showed the same results; therefore it appeared unnecessary to further examine the right side for motile spermatozoa.

The findings on the left side (scrotal confinement) merit some explanation, since this condition has not yet been considered. Seven days after the operation motile spermatozoa were obtained from the head and tail of the epididymis and ductus deferens of one animal. The motility was slightly reduced at all levels. Extensive adhesions of the tract in one animal examined at this time probably accounts for the early loss of motility in the lower levels. Observations eight days after scrotal isolation show little variation from the first animal described. There is, however, marked change in the motility of the spermatozoa at different levels after 10 days. Eight animals were examined at this time. In one case only a few feebly motile sperm were found in the head of the epididymis. Seven showed live spermatozoa in the tail of the epididymis, and only five revealed live sperm in the ductus deferens. There was a reduction of motility at both levels in most preparations; however, the loss was slightly greater in the ductus deferens. Some feebly motile spermatozoa were recovered from the tail of the epididymis and ductus deferens of only one animal after 12 days. On the fourteenth day live sperm were observed only in the tail of the epididymis, but the motility was very slight. Six animals were killed and examined 16 days after isolation of the parts; in one case very slightly motile spermatozoa were found in the tail of the epididymis, and only dead sperm were recovered from all other levels.

The most outstanding feature shown in Table II is the early loss of motility in the spermatozoa when the epididymis and ductus deferens are confined in the abdomen. The longest time sperm remained motile in such instances was 8 days. However, when the epididymis and ductus deferens remained in the scrotum, live spermatozoa were found up to 16 days. In both cases the persistence of motility was longer in the tail of the epididymis than in any other part of the genital tract.

When Tables I and II are compared, it will be noticed that the reduction and complete loss of motility is first evident in the head of the epididymis, next in the ductus deferens, and finally in the tail of the epididymis. It should be remembered that this difference in loss of motility at different levels was observed when the genital tract was confined either in the abdomen or in the scrotal sac.

II. THE MOTILITY AND RESISTANCE OF SPERMATOZOA AT DIFFERENT LEVELS OF THE REPRODUCTIVE TRACT IN VITRO

In order to study the motility and resistance of spermatozoa at different levels of the reproductive tract *in vitro*, the desired levels were extirpated and the sperm removed as previously described. (Cohn, 1918) showed that the concentration of the sperm solution has some influence upon the persistence of motility; therefore approximately equal amounts of sperm were taken from each level. It was found that certain portions of the tract would yield about the same concentration of sperm when placed in from ½ to 1 cc. of the solution in which they were to be studied. For this reason, the portions actually used were as follows: one-fourth of the testis, one-half of the head, the whole body, one-third of the tail of the epididymis, and the entire ductus deferens. At intervals of from 15 minutes to 1 hour a drop of the sperm suspension was placed in a depression slide and examined for motile cells.

The spermatozoa thus obtained were studied under the following conditions: (1) normal motility in physiological saline solution (0.9 per cent); (2) in increased and decreased H-ion concentrations; (3) in various saline solutions at scrotal, abdominal, and room temperatures; (4) in normal physiological saline solution at 2° C.

Normal motility in physiological saline solution.—Since there is some controversy regarding the relative motility of spermatozoa in the testis and at different levels of the reproductive tract, it appeared necessary to study the normal motility in connection with this problem. Five animals were used in this study, and the normal motility of the spermatozoa from successive levels of the tract, when suspended in normal physiological saline solution (0.9 per cent) and immediately examined, was found to be as follows: testis *, head and body of the epididymis ***, tail of the epididymis ****, and ductus deferens ****. In support of the results thus obtained, the same motility was noted, upon immediate examination, in the observations to be described later.

In increased and decreased H-ion concentration.—Since carbon dioxide is constantly being formed by the metabolism of the sperm (Cohn, 1918), it was necessary to use a buffer solution in order to maintain a constant H-ion concentration throughout the experi-

ment. Standard buffer solutions of known H-ion concentration covering a range of pH 6.2 to 9 were used. The buffers were prepared according to the method of Clark and Lubs (1917). It is realized that such solutions are not isotonic with spermatozoa, but it was found that physiological saline solution made to a definite pH with an acid or base gave almost the same results; therefore isotonicity may be considered a minor factor when studying this phase of the problem. It must be remembered that a neutral solution has a pH of 7, and that any number greater than 7 represents alkalinity, while less than 7 indicates acidity.

TABLE III

MOTILITY AND RESISTANCE IN INCREASED AND DECREASED H-ION CONCENTRATION
(Temp. = 25° C.)

pH	Testis	Head of Epididymis	Body of Epididymis	Tail of Epididymis	Ductus Deferens
6.2	15 min.	30 min.	2 hr.	2½ hr.	15 min.
6.4	15 min.	30 min.	2 hr.	3 hr.	15 min.
6.6	15 min.	2 hr.	2 hr.	3 hr.	15 min.
6.8	15 min.	3 hr.	3 hr.	3 hr.	15 min.
7	15 min.	3 hr.	6 hr.	6 hr.	30 min.
7.2	30 min.	3 hr.	6 hr.	6 hr.	30 min.
7.4	1 hr.	7 hr.	7 hr.	8 hr.	1 hr.
7.6	4 hr.	9 hr.	9 hr.	9 hr.	1 hr.
7.8	4 hr.	9 hr.	8 hr.	9 hr.	2 hr.
8	4 hr.	3 hr.	4 hr.	8 hr.	2½ hr.
9	45 min.	2 hr.	2 hr.	3 hr.	45 min.

Table III shows the results of observations on 22 animals. Two animals were used for each pH solution, thus obtaining four testes, epididymides, and ductuli deferentes. The time given represents the average duration of motility on the part of the last sperm to become quiescent.

These observations show that the resistance of the sperm to acidity and alkalinity increases from the testis to the tail of the epididymis, and decreases after they pass into the ductus deferens. A very slight increase in the H-ion concentration (acidity) has a marked destructive effect on sperm motility at every level, while the same relative decrease in H-ion concentration (alkalinity) is less deleterious. The spermatozoa in the testis and ductus deferens are more rapidly affected by acidity than alkalinity in comparison to the sperm at

any level of the epididymis. However, from the results obtained it is evident that the spermatozoa in the tail of the epididymis are more resistant to either increased acidity or alkalinity than those in any other part of the reproductive tract.

Motility and resistance in normal physiological saline, Tyrode's, and Ringer's solutions at scrotal, abdominal, and room temperature.— The observations *in vivo* show that when an isolated epididymis is confined in the abdomen the spermatozoa lose their capacity for motility in about one-half the time as when the epididymis remains in the scrotum; this was likewise observed by Moore, Heller, and others.

It will be recalled that when the epididymis was kept in the abdomen the sperm in the head of the epididymis were immobilized first, then those in the ductus deferens, and finally those in the tail of the epididymis. Such observations show that some sperm are more resistant than others to abdominal temperature. However, when the epididymis was treated in the same manner but left in the scrotum, there was a similar inactivation of the spermatozoa even though the persistence of life was correspondingly longer. In view of this situation it was thought that perhaps a study of the sperm from each level at abdominal and scrotal temperatures *in vitro* might show whether or not some sperm were more resistant than others to abdominal temperature.

The abdominal and scrotal temperatures of several rats were measured. At room temperature (25° C.) the average for the former was found to be 34° C. while the latter was 31° C. Other workers have reported comparable differences between abdominal and scrotal temperatures in the rabbit and guinea pig.

Two constant-temperature ovens provided the means of obtaining the desired temperature. One was maintained at 34° C., which corresponded to abdominal temperature; and the other was kept at 31° C., corresponding to scrotal temperature. The spermatozoa were studied at room temperature for the purpose of comparison; and by using different standard saline solutions, it was thought that a more correct average could be obtained.

The results obtained are recorded in Table IV, and the time given refers to the average duration of motility on the part of the last

sperm to become non-motile. The number of animals used for each saline solution is indicated.

The data given in Table IV show that a temperature corresponding to abdominal temperature inactivates the spermatozoa quicker

TABLE IV

MOTILITY AND RESISTANCE IN NORMAL PHYSIOLOGICAL SALINE, TYRODE'S, AND RINGER'S SOLUTIONS AT SCROTAL, ABDOMINAL, AND ROOM TEMPERATURE

(In hours)

Temperature	Testis	Head of Epididymis	Body of Epididymis	Tail of Epididymis	Ductus Deferens
In Tyrode's solution (average for 5 animals)					
Scrotal	7.5	9.5	11.5	13	12
Abdominal	2.5	7	7	12	9
In Ringer's solution (average for 5 animals)					
Scrotal	5	8.5	9.5	10.5	10
Abdominal	2	7	7	8	8
In normal saline solution (0.9 per cent)					
Scrotal	5	8	8	10	10
Abdominal	5	7	7	9	7
Total average for 14 animals for foregoing solutions					
Scrotal	5.8	8.7	9.7	11.1	10.7
Abdominal	3.2	7	7	9.7	8
In normal saline solution (0.9 per cent) at room temperature (25° C.) (average for 5 animals)					
	6	8	8	12.5	9.5

than scrotal temperature. The results also prove that the resistance of the sperm increases from the testis to the tail of the epididymis, and decreases slightly after they pass into the ductus deferens. This progressive degree of resistance is likewise manifested at room temperature (25° C.). By comparing the persistence of motility in normal saline solution at room temperature with that of scrotal and

abdominal temperatures, it will be noticed that there is only a slight difference in the length of retention of motility between scrotal and room temperatures and a greater difference between scrotal and abdominal temperatures. One would expect the spermatozoa to live considerably longer at 25° C. than at 31° C. But since this is not the case, it appears that just as soon as the temperature exceeds scrotal or normal temperature the increase in destructive effect on sperm life is greatest per degree.

Thus far in the study we have seen that the resistance of spermatozoa to acidity and alkalinity and to room, abdominal, and scrotal temperatures increases from the testis to the tail of the epididymis but decreases in the ductus deferens.

In normal physiological saline solution at 2° C.—Following the foregoing study, one would naturally ask what effect abnormally low temperatures have on spermatozoa at different levels of the reproductive tract. To answer this query, 8 animals were used. The spermatozoa were obtained as already described, placed in normal saline solution, and kept in a refrigerator at a constant temperature of 2° C. The average length of persistence of motility for each level is as follows: testis, 75 hours; head of epididymis, 14 hours; body of epididymis, 10 hours; tail of epididymis, 10 hours; ductus deferens, 8 hours.

These findings show almost the opposite of the results obtained in Tables III and IV. It was noticed there that the resistance of the sperm to alkalinity, acidity, etc., increased from the testis to the tail of the epididymis and decreased in the ductus deferens. At low temperatures the spermatozoa in the testis are very resistant, and the length of time of motility is exceedingly long. The corresponding persistence of motility at the other levels of the reproductive tract is hardly comparable. However, excluding the testis, it will be seen that the spermatozoa in the head of the epididymis are more resistant than those in the body or tail of the epididymis or ductus deferens. In summary it may be said that the resistance of the spermatozoa to low temperatures decreases from the testis to the ductus deferens. This great variation between the length of persistence of motility in the testis and the other parts will be considered in the following paragraphs.

DISCUSSION

The facts established concerning the resistance of spermatozoa to abdominal temperature, alkalinity, acidity, and low temperatures, as well as their normal motility in the reproductive tract, demand further discussion.

It is known that mammalian spermatozoön in general gain in intensity of motility as they pass down the epididymal canal, but several points of controversy are associated with spermatozoan motility in the testis and epididymis. Tournade and Regaud (1911), Tournade (1913), and Benoit (1926) have maintained that spermatozoa found within the testis tubules have not acquired the power of motility but only acquire it after being stored in the epididymis. Anderson (1922) states that little or no activity is manifested by the spermatozoa as they pass down through the epididymis and sperm cord. On this point, Tournade (1913) found that rat spermatozoa taken from the head of the epididymis were non-motile nine times out of ten, whereas in the tail of the epididymis and ductus deferens they were very motile and showed no difference in vigor of movement. Similar results were observed in the dog, cat, guinea pig, and rabbit. Contrary to some of these findings, Young (1929) has obtained feebly motile spermatozoa from the testes of the bull, ram, guinea pig, and rat. Moore (1928) has also observed motile sperm in the tubules of the rat's testis.

To study this point, the testes of 5 rats were used; and in every case motile spermatozoa were recovered. Similarly, very motile sperm were observed in the head of the epididymis as well as at other levels. It was shown that there was a progressive increase in vigor of motility from the testis to the tail of the epididymis. Likewise, one can note the increase in the quantity of sperm that become motile as the tail of the epididymis is approached. Such observations were supported by the same results in nearly 50 animals used in experiments other than especially for this study.

It is often stated that the spermatozoa acquire motility only through the action of the epididymal secretions; but since motile sperm were found in the testis, it appears that motility is not entirely dependent upon the epididymis. However, the function of this or-

gan has not yet been fully determined, and such a discussion is not the purpose of this paper.

The resistance of spermatozoa at different levels of the reproductive tract to abdominal temperature *in vivo* is interesting. The deleterious effect of abdominal temperature on spermatogenic tissue and spermatozoan activity has been observed by Moore (1928 and earlier studies) and his collaborators, and I have but to confirm their results. It may be pointed out, however, that their studies pertained to spermatozoal life in the epididymis as a whole rather than at any specific level. Moore found that spermatozoa of the rat retained their capacity for motility for approximately 14 days in an epididymis which was isolated in the scrotum when both testes had been removed. But Heller (1928) showed that this period of persistence of motility was reduced to 5 or 6 days when the epididymis was confined within the abdomen. Similar results were obtained by Oslund (1924), Lawrence (1926), Benoit (1926), and others.

In the experiments recorded in this paper it was found that when the epididymis and ductus deferens were confined in the abdomen or in the scrotum the spermatozoa at different levels were unequally affected. When subjected to abdominal temperature (*in vivo*), the sperm in the head of the epididymis retained their capacity for motility for a maximum period of 6 days, in the ductus deferens for 7 days, and in the tail of the epididymis for 8 days. One case extended motility to the eighth day in the ductus deferens.

In order to appreciate the results obtained when the epididymis and ductus deferens are allowed to remain in the scrotum, it must be remembered, as shown by previous results, that the period of persistence of motility is considerably increased by the lower temperature in the scrotal sac (*supra*). In scrotal confinement the spermatozoa in the head of the epididymis lived for 10 days, in the ductus deferens for 12 days, and in the tail of the epididymis for 16 days.

From the results obtained in the abdominally confined epididymis and ductus deferens, it appears that abdominal temperature affects the sperm in the head of the epididymis first, then those in the ductus deferens, and finally those in the tail of the epididymis. But from the findings in scrotal retention of the epididymis and

ductus deferens, one cannot necessarily say that abdominal temperature has such an effect, since the spermatozoa manifested a similar loss of motility even though their period of existence was extended. It does seem, however, that the younger sperm (those in the head of the epididymis) are least resistant of any in either case. This would be comparable with the results obtained by Young (1927) for the guinea pig. He found that the sperm in the testis were less resistant to heat application than those in the epididymis; that is, the younger spermatozoa were killed first. Nevertheless, it will be noticed that the sperm in the tail of the epididymis survive longer than those in the ductus deferens. In answer to this, it is perhaps possible that the spermatozoa reach a maximum resistance in the tail of the epididymis, then weaken as they pass into the ductus deferens. One would not expect this to be the case, since the sperm in the ductus deferens are the ones that are normally ejaculated for fertilization. However, it will be shown later that the peak of resistance is reached in the tail of the epididymis and not in the ductus deferens.

The resistance of spermatozoa at different levels of the reproductive tract *in vitro* offers some controversy in regard to higher temperatures. For studying the increased resistance of spermatozoa as they pass down the epididymal canal, considerable attention has been directed to high temperature as a measure of sperm strength. Stigler (1918) says that through the action of the epididymis the spermatozoa acquire a heat tolerance. He maintains that the resistance of the sperm to heat increases from the testis to the tail-end of the epididymis, and that the intensity of motility as well as resistance to heat increases after the spermatozoa leave the epididymis. Young (1927) noted that there was a progressive disappearance of the sperm from the head to the tail-end of the epididymis in the guinea pig when heat was applied to the scrotal sac. However, a more recent study by Young (1929) on the heat resistance of sperm removed from various levels of the epididymis in several mammals indicates that the spermatozoa do not become heat resistant as they pass down this duct. Von Lanz (1927) believes that the epididymal secretions increase movement and persistence of motility. Anderson (1922) observed that spermatozoa were very

sensitive to different chemicals and to temperature changes. When horse semen was kept at 22° C., the sperm were all dead after an average of 6 hours. However, when the temperature was raised to 38° C., there was a total loss of movement in 4 hours on the average. Anderson noted that some sperm were more resistant than others to high temperature.

Heat resistance alone did not appear to be the only criterion as a measure of sperm strength, and for that reason other methods were used. So many important biological factors depend upon a limited range of the H-ion concentration for proper function that alkalinity and acidity offered an inviting means of measuring the resistance of spermatozoa at different levels of the reproductive tract. It was found that the resistance of the sperm to alkalinity and acidity increased from the testis to the tail of the epididymis and decreased in the ductus deferens. The buffer solutions in which the spermatozoa were studied covered a range of from pH 6.2 to 9. In such solutions the sperm at all levels of the tract were more sensitive to acidity than alkalinity, but those in the testis and ductus deferens were less sensitive to increased alkalinity in proportion to the sperm at any level of the epididymis. However, it was found that the spermatozoa in the tail of the epididymis were more resistant to either increased acidity or alkalinity than those in any other part of the reproductive tract.

The differences in resistance of the spermatozoa to abdominal and scrotal temperature *in vitro* merits some discussion. It will be recalled that the temperature in the abdomen is about 3° C. higher than the intrascrotal temperature. The study *in vivo* shows that this seemingly small increase in temperature is sufficient to reduce the life of spermatozoa to about one-half of their normal period of existence when the genital tract is elevated from the scrotum into the abdomen. It is not hard to understand why such a slight increase in temperature inactivates these highly specialized cells so quickly when we consider how other cells in the body are affected by febrile conditions.

In vitro, it was found that when the sperm taken from any level of the reproductive tract were kept at a temperature corresponding to abdominal temperature they were killed approximately 2 hours

sooner than at intrascrotal temperature. The spermatozoa removed from the testis were unquestionably more sensitive to abdominal temperature than those at any other level. The higher temperature inactivated them in less time, as compared to the sperm in the epididymis and ductus deferens. Again, the spermatozoa in the tail of the epididymis showed more resistance to abdominal temperature than did those in other portions of the tract.

When comparing the time results just stated with those obtained *in vivo*, it should be remembered that while the spermatozoa are stored in the male tract they are dormant and consequently at a low metabolic level and that therefore their life-span is a matter of many days. However, as soon as they are removed from those ducts and placed in an appropriate solution, or released at copulation, they become vigorously motile and undergo metabolic activity tending to a more rapid death, so that their life-span is a matter of only a few hours. Likewise, when the spermatozoa are ejaculated into the uterus at coitus, their persistence of motility is somewhat longer than when they are kept in physiological saline solutions. For the rat the maximum time of motility after copulation was found to be 17 hours, while in the guinea pig this period was increased to 41 hours (Yochem, 1929).

Let us consider the effects of low temperature on the spermatozoa at different levels of the reproductive tract. Spallanzani (1785), and later Prévost and Dumas (1824), called attention to the fact that low temperature prolonged the life of spermatozoa. More recently Ivanoff (1922) has shown that sperm should be kept at 1°–2° C. when preserving them for artificial insemination. Wolf (1921) was able to hold rabbit spermatozoa, taken from the epididymis, in a state of potential motility for 9 days. This was accomplished by using a properly balanced and buffed physiological saline solution which had an H-ion concentration of 7.4, with a temperature near 2° C.

In the preceding discussion we noted that the resistance of the spermatozoa increases from the testis to the tail of the epididymis where a maximum is reached, then decreases in the ductus deferens. It was observed, however, that almost opposite results were obtained when the sperm were subjected to a very low temperature,

2° C. Here, the spermatozoa removed from the testis remained alive for about 75 hours, while the sperm at other levels of the tract retained motility for approximately 10 hours. There was a slight decrease in resistance from the head of the epididymis to the ductus deferens. This great variation between the length of persistence of motility in the testis and the other parts may perhaps be answered on the basis of the rate of motility. The sperm in the testis are only slightly motile as compared to those in the epididymis and ductus deferens. It will be remembered that low temperatures inhibit motility (Spallanzani and others); consequently, the spermatozoa of least motility would be least affected by decreased temperatures. A summary of my own observations on this point is of significance here. When the sperm suspension was examined after it had remained at 2° C. for a short time, it was found that the sperm were practically non-motile when first placed on the stage of the microscope but that after remaining a minute or two on the warm stage the motility increased considerably. As the period of time at 2° C. increased, the gradual disappearance of motility was very evident in the sperm taken from the epididymis or ductus deferens, while those from the testis showed little variation. It is probable, therefore, that the most inactive sperm are least affected by low temperature, as is evident by their consistence in motility. This also appears to be the case in the seminal canal, where the sperm in the head of the epididymis lived for a slightly longer period than those in the body or tail, or in the ductus deferens. However, it should be remembered that under all conditions, other than low temperature, the resistance of the spermatozoa increased from the testis to the tail of the epididymis and decreased in the ductus deferens.

SUMMARY AND CONCLUSIONS

1. When both testes are removed and the spermatozoa in the head and tail of the epididymis and ductus deferens are isolated with ligatures and confined within the abdomen, the maximum period of survival of motility at each level is as follows: tail of the epididymis, 8 days, ductus deferens, 7 days; and head of the epididymis, 6 days.

2. When isolated, as just indicated, and retained in the scrotal

sac, the period of persistence of motility at each level is increased to the following times: tail of the epididymis, 16 days; ductus deferens, 12 days; and head of the epididymis, 10 days.

3. The spermatozoa in the tail of the epididymis show a tendency to retain their capacity for motility longer than those at any other level; they are more resistant when subjected either to abdominal temperature or when allowed to remain in the scrotum. Moreover, the spermatozoa in the ductus deferens show a slightly less marked resistance than do those in the tail, while the spermatozoa in the head of the epididymis are least resistant under both conditions.

4. The deleterious effects of abdominal temperature on spermatozoan life is again demonstrated. If both testes are removed and the epididymis is kept within the abdomen, such normal abdominal temperature is sufficient to reduce the life of the spermatozoa to approximately one-half their period of existence when allowed to remain in the scrotum. This confirms the results of Moore, Heller, and others.

5. There is a noticeable difference in the rate of motility of spermatozoa when removed from different levels of the reproductive tract and placed in a normal physiological saline solution (0.9 per cent). The intensity of motility increases from the testis to the tail of the epididymis where a maximum is attained; in the ductus deferens, which always contains millions of spermatozoa, there is no recognizable further increase in their activity. Likewise, there is a progressive increase in the relative number of motile spermatozoa until a maximum is reached in the tail of the epididymis.

On the basis of the rate and relative abundance of motile sperm, the following motility rating is estimated for each level: testis *, head and body of the epididymis ***, tail of the epididymis ****, and ductus deferens ****.

6. The resistance of the spermatozoa to alkalinity or acidity increases from the testis to the tail of the epididymis, then decreases in the ductus deferens.

7. When spermatozoa are removed from successive levels of the reproductive tract, placed in normal physiological saline (0.9 per cent), Tyrode's or Ringer's solutions, and kept at a temperature corresponding to abdominal (34° C.) or scrotal temperature (31° C.),

there is a comparable difference in the persistence of motility. The cells from each level live an average of about 2 or 3 hours longer at the scrotal temperature than at abdominal temperature. However, at either abdominal or scrotal temperature there is an increase in resistance from the testis to the tail of the epididymis, and a decrease in the ductus deferens.

8. If spermatozoa are removed from successive levels of the reproductive tract, placed in normal physiological saline solution (0.9 per cent) and kept at a temperature of 2° C., there is a decrease in resistance from the testis to the tail of the epididymis and ductus deferens. Since low temperatures inhibit motility, it is believed that the spermatozoa of lowest motility are least affected by very low temperatures. Indeed, the loss of motility at each level was in the reverse order of the normal increase in motility down the reproductive tract.

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THE ACTION OF ULTRA-VIOLET RAYS ON ARBACIA EGG PROTOPLASM

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IT IS a well-known fact that ultra-violet rays have a stimulating action on various types of living substance. But at the present time, the nature of the effect that these rays have on the protoplasm is but little known.

One of us (Heilbrunn) has shown that when sea-urchin eggs are stimulated to division by any one of a wide number of reagents or treatments, the protoplasm is always gelled, that is to say, it undergoes a very pronounced increase in viscosity. (For references, see Heilbrunn, 1928.) Now, up until the present, it has not been shown that ultra-violet rays produce either this or any other physical effect on the protoplasm of sea-urchin eggs. One purpose of our investigation was to discover whether ultra-violet rays, acting for optimal times necessary to produce stimulation to cell division, did actually cause changes in the viscosity of the protoplasm.

It is believed that the protoplasmic viscosity increase which accompanies or follows stimulation is the direct result of a specific reaction in the protoplasm (see again Heilbrunn, 1928). This reaction is in some ways comparable to the reaction which occurs when blood is coagulated. It takes place in two main stages, the first of which requires calcium. Because of this need for calcium in the first stage of the reaction, it is often possible to inhibit the reaction by the removal of calcium. We were led, therefore, to inquire into the possible effects of a lack of calcium on the stimulating power of ultra-violet rays for the sea-urchin egg.

The present investigation is therefore in two parts. In the first place, the viscosity of the protoplasm was measured at various times following exposure to ultra-violet radiation. Secondly, eggs were radiated in the absence of calcium in order to discover whether

the presence or absence of this element had any effect on the subsequent behavior of the radiated eggs. The work was done at the Marine Biological Laboratory at Woods Hole, and our thanks are due to the director of the Laboratory for the privileges we enjoyed there.

Lillie and Baskervill (1922*b*) studied the effect of ultra-violet rays on sea-urchin eggs, and they were able to show that the radiation caused membrane elevation. Longer exposures induced cytolysis. When the radiated eggs were treated with hypertonic solutions, they apparently showed a slightly greater tendency to begin development than the unradiated controls. In Lillie and Baskervill's experiments, the eggs did not ordinarily segment following radiation unless they received an after-treatment with hypertonic sea water. However, one of us (Young) has been able to obtain somewhat better results in this regard, and her experiments have shown both cleavage and development of gastrulae in eggs which were radiated but received no after-treatment with hypertonic sea water. These experiments will be published in detail elsewhere.

The present study is an attempt to investigate the stimulating action of ultra-violet rays. It is not concerned at all with the determination of the actual wave length of the rays which are most potent in causing the stimulation. But, granting that ultra-violet rays do stimulate the eggs, we were interested in discovering what their effect might be on the protoplasm. Our method of radiating the eggs was then essentially that of Lillie and Baskervill (1922*a* and *b*), and we used either the very lamp that they did or one closely similar to it. This is a mercury-arc quartz lamp, operating at about 110 volts and 4 amperes. The eggs were exposed in Syracuse watch glasses in less than 1 cc. of sea water (10 drops), and the distance of the bottom of the dish from the arc was approximately 24 cm. Experiment showed that an exposure of 1 minute was sufficient to produce a high percentage of membrane elevation in the treated eggs, and this length of exposure was used in practically all the experiments. For this short exposure, the temperature of the eggs did not rise appreciably. No precautions were therefore necessary to guard against heating.

When eggs are radiated for 1 minute, there is often considerable

variation in the results that are obtained with different lots of eggs, or even with the same lot of eggs at different times. When sea-urchin eggs lie in sea water, they give off some of their pigment to the surrounding medium. Probably this pigment is capable of absorbing ultra-violet rays. At any rate, eggs which have stood in sea water do not appear to be as susceptible to the radiation as are freshly immersed eggs or eggs which have been washed free of the so-called "egg water." In addition to the variation which is due to biological factors such as individual differences in eggs or lots of eggs, and differences in the amount of pigment in the fluid over the eggs, there is also some variation of purely physical origin. The intensity of the light given off by the mercury arc lamp doubtless varied from time to time, in spite of the fact that care was taken to keep conditions as constant as possible. Because of the variations in response of the eggs, it is scarcely possible to combine results from different experiments. In every case, ultra-violet radiation which was effective in stimulating the eggs caused a sharp increase in protoplasmic viscosity, but this viscosity increase in some instances appeared much more rapidly than in other instances. In measuring protoplasmic viscosity by the centrifuge method, it is customary to make a series of tests in which the protoplasm to be tested is centrifuged for varying lengths of time in order to determine just how many seconds of a given centrifugal treatment are necessary to produce a given degree of granular movement. But in our studies this is not possible. The viscosity of the protoplasm is constantly changing after exposure. One can make only one test at a given time; and because of the variations in the response, it is not possible to make a series of tests on different lots of eggs all given the same treatment. Thus, 5 minutes after a 1-minute exposure, one lot of eggs may have a very different protoplasmic viscosity from another lot apparently given identical treatment.

The normal viscosity of the granule-free protoplasm of the *Arbacia* egg at room temperature is approximately 0.03 in absolute units, or three times the viscosity of water (see Heilbrunn, 1928). In making centrifuge tests of the viscosity, a Bausch and Lomb hand centrifuge was used, and the high speed handle was turned one time per second. Under these conditions, a force 4,968 times

gravity was developed. The ordinary procedure was first to centrifuge normal untreated eggs for 30 seconds. These were then examined, and an attempt was made to fix a mental image of the appearance of these control eggs. Typically they showed a more or less complete stratification, with a granule-free or hyaline zone extending along about one-third of the diameter of the egg. It is not hard to retain this mental picture of eggs with a hyaline zone of this definite dimension. Later, when radiated eggs were centrifuged for varying lengths of time, it was quite possible to decide whether or not the degree of stratification attained was roughly more or less than that shown by these normal eggs after 30-second treatment. Now, if radiated eggs when centrifuged for 20 seconds show the same degree of stratification as the normal eggs centrifuged 30 seconds, their viscosity is two-thirds that of the normal, and is therefore 2 (in terms of water). Of course, no very delicate measurements can be made in this way. But the viscosity changes of the protoplasm are so pronounced that it is not necessary to apply methods of a high order of accuracy. This will be fully apparent from what follows.

One difficulty in making measurements lies in the fact that the interior protoplasm of the cell and the more peripheral region do not show the same viscosity changes at the same time. Immediately after radiation, the viscosity of the cortical protoplasm increases, whereas that of the interior decreases. The eggs centrifuged at such times show hyaline zones which are overcast with granules. No attempt was made to determine the viscosity of the cortical region of the cell, and the data presented concern only the main central mass of the protoplasm. Thus, in making viscosity determinations, attention was focused on the center of the cells.

Table I, which gives the results of only a single experiment, shows the viscosity changes following radiation for 1 minute. The room temperature during the experiment was about 22.6° C. The first column gives the time in minutes after exposure to rays was complete. The second column shows the approximate viscosity in terms of the viscosity of water.

In interpreting this table, it should be clearly understood that when a value is given as less than or more than a given quantity,

this does not mean that the proper value is slightly less or more than the value given. As previously explained, only one test was possible at a given time; and all this could show was whether the viscosity was less or more than a given value.

The results given in the table show clearly that, following radiation, there is a marked decrease in viscosity, followed by an even more marked increase in protoplasmic viscosity. The low viscosity persists for about 15 minutes. During this time there is probably an increase in the thickness of the cortical zone of higher viscosity.

TABLE I

VISCOSITY OF *Arbacia* PROTOPLASM FOLLOWING EXPOSURE TO ULTRA-VIOLET RAYS

Minutes after Radiation	Viscosity
2	More than 2
4½	About 2½
6	About 2
10	Less than 3
15	Less than 3
20	About 3 (or slightly more)
25	More than 3
27½	About 6
31	More than 7
35	About 9 (or slightly more)
45	More than 11
Control untreated	3

At any rate, after 15 minutes the viscosity of the interior protoplasm rises markedly. This rise in viscosity was always noted. The time relations, however, were not always the same. Sometimes viscosity increase began in less than 15 minutes after radiation, and the period of viscosity decrease might be shortened or might not appear at all. In one experiment the viscosity was about 9 when tested 30 minutes after radiation, and it rose no higher within the next 10 minutes.

All the experiments agree in showing a very definite viscosity increase following ultra-violet radiation, and this increase is a threefold or fourfold one.

The results with ultra-violet radiation are therefore consistent with the knowledge that has so far been gained, to the effect that every type of stimulation which is at all effective in inducing cell

division in the sea-urchin egg produces a marked increase in the viscosity of the protoplasm (see Heilbrunn, 1928, chap. xv). Moreover, it is worth noting that the general magnitude of the viscosity increase with ultra-violet radiation is the same as that which follows sperm entrance and the normal stimulus to cell division.

It must not be thought that the sea-urchin egg presents an isolated case. From a survey of the literature, one of us has been able to show that in various types of protoplasm, initiation of activity is accompanied or followed by a sharp rise in viscosity (Heilbrunn, 1928, chap. xvi). Indeed, this is true for every type of protoplasm that has been studied, whether plant or animal.

Now the increase in protoplasmic viscosity which is associated with stimulation is doubtless but one aspect of a number of changes, both physical and chemical, which are occurring in the protoplasm. In the work above cited, an attempt was made to show that the viscosity increase was the direct result of a specific reaction or series of reactions common to protoplasm in general. These reactions can be most readily studied when a cell is injured and the protoplasm flows out. But they may also take place throughout intact cells. Typically the reactions occur in two stages. In the first of these, calcium is necessary; and in the *Arbacia* egg the calcium causes a breakdown of pigment granules. The second stage requires no calcium. There is no need here to summarize again the various facts that have been learned about the reaction or reactions that we are considering.

The evidence is good that ultra-violet light produces in sea-urchin eggs the very type of reaction that we regard as so important. Lillie and Baskervill (1922*b*) reported that the ultra-violet light caused cytolytic changes. Now these cytolytic changes in themselves are the morphological aspect of the reaction we have described, a reaction which, for want of a better name, we may continue to characterize as an internal surface precipitation reaction. During cytolysis, the pigment granules of the *Arbacia* egg are broken down, and the egg becomes filled with a mass of tiny vacuoles.

If cytolysis is an internal precipitation reaction, it may also be thought of as occurring in two stages. During the first stage of the reaction calcium reacts with the pigment granule, with the resultant

formation of some substance which then causes the formation of vacuoles or films throughout the protoplasm. The question now arises as to how ultra-violet rays can play a part in such a reaction system. Experimental analysis directed toward finding an answer to this question proved surprisingly easy. It was very soon found that in the absence of calcium, ultra-violet rays had little or no effect on the protoplasm. As pointed out previously, the first visible effect of the rays is to cause a membrane elevation which is practically identical in appearance with that produced by the sperm on insemination. This membrane elevation is then followed either by division of the egg cell or, more generally, by cytolysis. But when eggs are previously treated with an oxalate solution for varying lengths of time, and are then rayed, they never show any trace of membrane elevation. The experimental results are clear cut and are very convincing. The oxalate solution removes most of the calcium from the egg cortex, or from the entire mass of the egg if the exposure is long enough; and it does this without serious injury. Eggs previously oxalated and then returned to sea water will show membrane elevation either when inseminated or when exposed to ultra-violet radiations.

The following three tables show results obtained on radiating oxalated eggs. In Tables II and III the eggs were kept in oxalated sea water for 1, 3, 5, 10, and 15 minutes and were then radiated. The oxalated sea-water was prepared by adding one part of M/4 ammonium oxalate to two parts of sea water. The first column gives the time the eggs remained in the oxalated sea water; the other columns show the percentages of oxalated and control eggs of the same lot which showed membrane elevation. Control eggs were in sea water and were radiated at the same time and under the same conditions as oxalated eggs.

In Table II the results are clear cut, although the controls do not always show as high percentages of membrane elevation following exposure to ultra-violet radiation as were found typically for 1-minute exposure under the conditions of the experiment. Table III gives further data of the same sort as Table II. In this second collection of data the controls show higher percentages of membrane elevation.

Tables II and III give the records of eight series of experiments. In all, forty lots of previously oxalated eggs were exposed to ultra-violet light under conditions shown to be optimal for the stimulation of the eggs to artificial membrane elevation. In thirty-eight cases

TABLE II

PERCENTAGE OF MEMBRANE ELEVATION IN OXALATED AND CONTROL EGGS (EXPERIMENTS A–D)

Time in Oxalate Solution (Minutes)	Oxalated Eggs Lot A	Control Lot A	Oxalated Eggs Lot B	Control Lot B	Oxalated Eggs Lot C	Control Lot C	Oxalated Eggs Lot D	Control Lot D
15	0	20	0	45	0	90	0.5	69
10	0	12	0	15	0	75	0	73
5	0	19	0	18	0	64	0	79
3	0	18	0	20	0	50	0	50
1	0	15	0	66	0	50	0	55

the radiation was without effect. In two experiments an occasional egg showed the appearance of a membrane—one egg in one experiment, two eggs in another. It is doubtful if these rare membranes owed their appearance to the radiation. Elevated membranes or,

TABLE III

PERCENTAGES OF MEMBRANE ELEVATION IN OXALATED AND CONTROL EGGS (EXPERIMENTS E–H)

Time in Oxalate Solution (Minutes)	Oxalated Eggs Lot E	Control Lot E	Oxalated Eggs Lot F	Lot F	Oxalated Eggs Lot G	Control Lot G	Oxalated Eggs Lot H	Control Lot H
15	0	65–75	0	60–75	0	90	0	87
10	0	65–75	0	60–75	0	89	0	88
5	2	65–75	0	60–75	0	93	0	72
3	0	65–75	0	60–75	0	83	0	89
1	0	65–75	0	60–75	0	88	0	91

more often, membranes which simulate elevated membranes appear now and then in untreated and uninseminated eggs. This is doubtless the explanation of the three eggs with apparent membranes out of over four thousand counted. We are surely safe in assuming that the treatment with oxalate completely prevents any appearance of membrane elevation when eggs are radiated. This is a very striking result. It is significant, moreover, that an exposure to the oxalate

of only 1 minute is sufficient to prevent all membrane elevation. Such a short treatment can scarcely remove all of the calcium from the egg, but it is presumably sufficient to remove the calcium from the egg cortex, and it is doubtless on the cortex that the rays act.

Of course there is the possibility that the treatment with oxalate injures the eggs and that it is this injury which prevents stimulation by ultra-violet radiation. It is easily shown that this can scarcely be true. When oxalated eggs are returned to sea water and are then radiated, they give the normal response to ultra-violet rays. This is clearly shown by the results recorded in Table IV.

TABLE IV

PERCENTAGES OF MEMBRANE ELEVATION IN OXALATED EGGS AFTER RETURN TO SEA WATER, AS COMPARED WITH NORMAL EGGS

Time in Oxalate Solution (Minutes)	Lot A 95 Minutes in Sea Water	Lot A Control	Lot B 95 Minutes in Sea Water	Lot B Control	Lot C 45 Minutes in Sea Water	Lot C Control	Lot D 60 Minutes in Sea Water	Lot D Control
15	50	95	60	85	92	98	47	90
10	32	95	88	85	76	98	50	90
5	35	95	68	85	96	98	35	90
3	45	98	78	85	98	98	46	90
1	44	98	86	85	87	98	46	90

The experimental results given in Table IV perhaps require some additional explanation. As before, eggs were oxalated by being placed in a solution containing two parts sea water and one part M/4 ammonium oxalate solution. They were left in this oxalated sea-water solution for varying times (15, 10, 5, 3, 1 minute) and were then transferred to normal sea water. They remained in this sea water either for 95 minutes (Lots A and B), for 45 minutes (Lot C), or for 60 minutes (Lot D). Following this period in sea water, the previously oxalated eggs were exposed to ultra-violet radiation in the usual manner for 1 minute along with control eggs from the same female. The results show, beyond the shadow of a doubt, that after return to sea water, the previously oxalated eggs regain their power of being stimulated to membrane elevation by ultra-violet rays. In most cases, it is true, the percentage of membrane elevation shown by eggs previously oxalated is somewhat less than that of the control. But in every instance a substantial percentage of the eggs did

show membrane elevation. As a matter of fact, in Lots B and C half of the tests showed practically the same percentage of membrane elevation in eggs previously oxalated and control eggs never so treated. We must conclude, therefore, that exposure to oxalate does not kill the eggs.

In the oxalate solution the eggs are relatively free from calcium, and no membrane elevation occurs. Merely by allowing the calcium to return to the eggs, the eggs can be made susceptible again to ultra-violet radiation.

It may be noted that if the calcium is removed from *Arbacia* eggs by two or three washings in isotonic sodium-chloride solution, the eggs also become incapable of stimulation by ultra-violet rays. This method of removing calcium is not as convenient as the oxalate method, and it also produced more injury, presumably because of the removal of magnesium along with the calcium.

The question may now with justice be asked as to how the ultra-violet radiation produces the membrane elevation. The lifting of the membrane is a physical process and requires a physical interpretation. Some years ago one of us (Heilbrunn, 1913, 1915) proposed a theory of membrane elevation according to which the phenomenon was primarily due to a lowering of the surface tension. Is it possible to explain the action of ultra-violet rays on the basis of this theory? The answer is yes. At any rate, we can offer a formal explanation which will fit the facts. It has been shown that the ultra-violet rays cause an internal surface precipitation reaction. As pointed out previously, this reaction occurs in two stages. The first stage requires calcium, and results in the formation of a substance which has been called "ovothrombin." This substance, ovothrombin, is apparently very surface active, for it accumulates in the surface film of its solutions (see Heilbrunn, 1928, p. 227). We could therefore with justice conclude that ultra-violet rays by the production of ovothrombin do actually induce a lowering of the surface tension of the surface membrane of the *Arbacia* egg.

But it must not be supposed that this is the only possible interpretation. One characteristic of an internal surface precipitation reaction is the production of numerous vacuoles or vesicles in the cytoplasm. Perhaps the formation of these vesicles in the cortex

of the egg pushes the vitelline membrane away from the rest of the protoplasm. This alternative view might indeed serve as a more or less general explanation of membrane elevation, for the reagents which cause elevation of the membrane quite generally cause cytolysis or an internal surface precipitation reaction after a sufficiently long exposure. It was thought worth while to discover whether or not the membrane-elevating action of other types of treatment also depended on the presence of calcium. A few experiments were tried with toluol, and it was clearly shown that this reagent can produce high percentages of membrane elevation in the absence of calcium. Thus, in one experiment it was possible to obtain 96 per cent of perfect membrane elevation in oxalated solutions. The eggs were treated with toluol in the presence of oxalate, and they were then transferred to oxalated sea water.

The fact that toluol can produce membrane elevation in the complete or rather almost complete absence of calcium might be taken as evidence in favor of the surface-tension theory as opposed to the alternative theory that we have proposed. But in our opinion it is not conclusive evidence, for it is quite possible that in some conditions the surface precipitation reaction can occur in the absence of calcium. As a mere matter of observation, however, it is worth noting that membrane elevation can be incited by toluol in the absence of calcium, whereas this is not possible in the case of ultra-violet radiation.

It is not our main purpose, however, to consider theories of membrane elevation. We are concerned primarily with the action of ultra-violet rays as stimulants to protoplasmic activity. This they certainly are, both in the case of sea-urchin eggs and also in the case of many other types of protoplasm. It is important, therefore, that stimulating doses of ultra-violet radiation produce the typical viscosity increase which is now definitely known to accompany stimulation in various types of protoplasm by various agents. Our theory of the nature of stimulation is thus strengthened by direct additional evidence. Moreover, for ultra-violet rays it has certainly been shown that, in the sea-urchin egg at least, the effect of the rays is dependent on the presence of calcium; and this gives us very good reason to believe that the stimulating action of ultra-violet rays and

the increase in protoplasmic viscosity caused by them is primarily due to a form of internal surface precipitation reaction of the type previously shown to exist so generally in various types of protoplasm.

SUMMARY

1. Exposure to ultra-violet rays sufficient to produce stimulation in *Arbacia* eggs causes, in every case, an increase in protoplasmic viscosity.

2. Typically, the interior of the cell is at first more fluid following treatment with ultra-violet radiation. The increase in viscosity begins in the cortical region and gradually extends inward.

3. The viscosity increases to about three or four times its original value.

4. The stimulating action of ultra-violet rays is dependent on the presence of calcium. Oxalated eggs show no stimulation effects. Such eggs are not injured and can be made to recover their power to be stimulated on the addition of calcium.

5. The results are in thorough agreement with the theoretical views of Heilbrunn (1928), according to which stimulation of protoplasm is generally associated with a viscosity increase, an increase which is dependent on a peculiar reaction which in its early stages regularly involves calcium.

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RECONSTITUTION OF LATERAL PIECES OF PLANARIA DOROTOCEPHALA AND PLANARIA MACULATA

(Twenty-two figures)

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ALTHOUGH the reconstitution of planarians has been extensively investigated in various species and by numerous investigators, comparatively few data are available concerning the reconstitution of pieces from the lateral regions of the body. The data which exist, however, indicate that such pieces show certain interesting conditions as regards polarity. Morgan (1898, 1903) described several cases of reconstitution of lateral marginal pieces in which the "long axis of the new head" was "at right angles to the long axis of the original worm." He also noted that long, narrow marginal strips might give rise to more than one head on the median cut surface. Child (1915, pp. 163–64, Figs. 94–98) found that in lateral pieces representing half the width of the body the position of the new head varied with length of piece. In the longer pieces of one-half body-width the head arose from the anterior cut surface; in shorter pieces it might arise in part from the anterior and in part from the median cut surface; and in still shorter pieces, particularly in triangular pieces with apex near the median line and a very short base at the lateral margin of the body, it frequently arose from the median end of the piece, the apex of the triangle. He considered that in these cases the new polar axis differed from the old by 90°. Olmsted (1918) performed further experiments with essentially similar results but concluded that "the original polarity has not been fundamentally changed" in the cases in which the head develops from the median cut surfaces.

In the light of the observed facts, the basis for this conclusion is not entirely clear, but is perhaps the failure of such pieces to develop posterior ends from the original lateral margin of the body. It is difficult to understand how such failure affords adequate

grounds for the conclusion that an axis which differs visibly by 90° from the original axis is still the same. The lateral margin of the planarian body is a highly specialized region containing little, or in extreme lateral regions no, parenchyma and cannot be expected to develop a posterior end unless cut rather deeply. If, however, the whole marginal region is removed by a deep cut after development of the head from the median cut surface, the piece remains tailless. In this respect it does not differ from other very short pieces possessing a head (Child, 1911*b*).

Aside from the question whether a change of 90° in direction of axis does or does not constitute a change in polarity, it is of interest to analyze further, if possible, the factors determining such change.

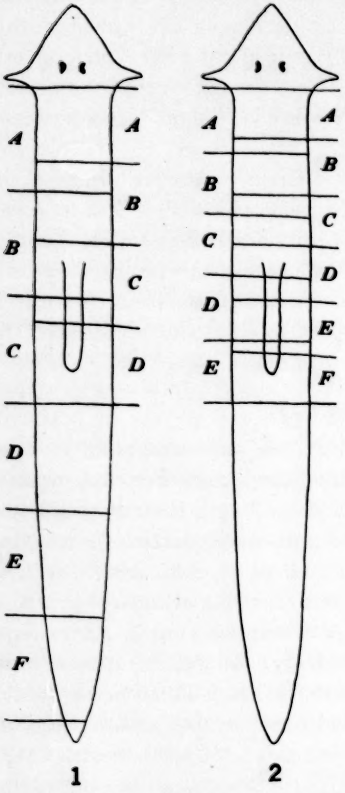
The present paper records data concerning the positions of anterior ends in one-half transverse pieces and pieces from the region lateral to the nerve cord, with respect to length of piece, level of body, and size or physiological age of animals.

MATERIAL AND METHOD

Both *Planaria dorotocephala* and *P. maculata* were used as material, the former more extensively than the latter. For each experiment individuals of approximately a given length from a stock kept in the laboratory under uniform conditions were selected, the lengths used being 8–10 mm., 16–18 mm., and 20–22 mm. for *P. dorotocephala*, and 16–18 mm. and 20–22 mm. for *P. maculata*.

In certain series made for purposes of comparison, the pieces represented one-half the width of the body. All other experimental pieces represented the region lateral to the longitudinal nerve cords, one-fifth to one-fourth the total width of the body, and were of different lengths: one-third (Fig. 1, left side, *A–C*), one-fourth (Fig. 1, right side, *A–D*), one-fifth (Fig. 2, left side, *A–E*), one-sixth (Fig. 2, right side, *A–F*), of the first zooid which in large individuals of *P. dorotocephala* represents about the anterior half of the body-length after removal of the head. Series of lateral pieces representing one-sixth of the entire body-length were also prepared (Fig. 1, left side, *A–F*), chiefly for the purpose of determining whether the posterior zooid region showed any marked differences from the anterior region. The pieces were obtained by first cutting transverse

pieces of the desired length and removing the lateral margins from these. The pieces were prepared in lots of forty to one hundred each, each lot representing pieces of a certain length from a certain body level. The experiments included a total of some eight thousand pieces.



FIGS. 1 AND 2.—Diagrammatic outlines indicating approximate body-levels of the different series of pieces. Fig. 1, *A–C*, left side, one-third pieces of first zooid; *A–F*, left side, one-sixth pieces of whole body; *A–D*, right side, one-fourth pieces of first zooid. Fig. 2, *A–E*, left side, one-fifth pieces of first zooid; *A–F*, right side, one-sixth pieces of first zooid.

In the case of *P. dorotocephala*, the pieces were examined and results recorded 10 days after section. Although the development of the head is not always completed within this period, eyes or cephalic lobes occasionally appearing later, it is sufficiently advanced to permit determination of its position with respect to the original body-axes; in fact, its position can be determined with greater certainty before the development of pigment in the new tissue obscures the boundary between old and new tissue. In *P. maculata* reconstitution is somewhat less rapid, and records were made after 14 days.

The death-rate is very high in all the experiments. A considerable proportion of the deaths takes place during the period of contraction of the cut surface after section. Apparently the delay in closure of the cut surfaces, in consequence of their relatively great extent, and the frequent rupture of the body-wall at other points, in conse-

quence of the strong contraction of the cut surfaces, contribute to this high mortality. A few experiments made with a modified Ringer's solution, which prevents contraction of the cut surfaces, showed no decrease in death-rate. Some deaths occur during reconstitution.

THE FORMS RESULTING FROM RECONSTITUTION OF LATERAL PIECES

In the lateral pieces anterior and posterior cut surfaces are, of course, relatively small as compared with the median cut surface. Wound contraction decreases the angle between the terminal and median cut surfaces, and often in the shorter pieces brings the three surfaces almost or quite into the same plane, so that the original lateral margin of the piece becomes convex, even semicircular, in outline.

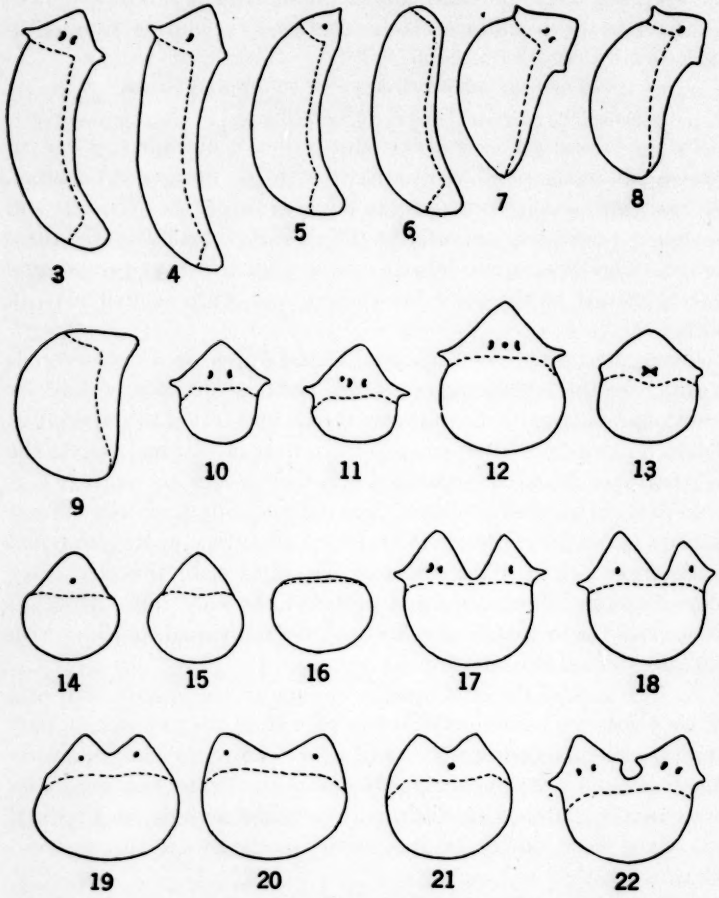
For present purposes the points of chief importance in the reconstitution of the lateral pieces are the position of the new head, or anterior end, and the direction of its axis in relation to the original longitudinal axis of the piece. With reference to these points the reconstituted forms are separated into four groups, as follows:

I. A head, normal or more or less differentially inhibited in form (Child, 1921), arises from the anterior cut surface of the piece and a posterior end from the posterior cut surface; or the piece may remain acephalic and develop a posterior end only (Figs. 3–6). In these cases the longitudinal axis of the new individual coincides with the longitudinal axis of the piece.

II. The axis of the new head is oblique to the longitudinal axis of the piece, the head developing in part from the anterior, in part from the median cut surface; and a new posterior end is usually absent (Figs. 7–9). The forms with new posterior end may gradually straighten out, so that the axis finally coincides with the longitudinal axis of the piece; but in the absence of a posterior end this does not occur.

III. A single head ranging in form from normal to anophthalmic, or in some cases only a slight outgrowth of new tissue, develops chiefly from the median cut surface with axis at right angles to the longitudinal axis of the piece, and the posterior end is absent (Figs. 10–16). In a small number of the shorter pieces the new tissue is

limited to healing of the cut surfaces without actual outgrowth of any kind. Such pieces are probably apolar or almost apolar, but in



FIGS. 3–22.—Forms which develop from lateral pieces. Figs. 3–6, anterior end at anterior end of piece. Figs. 7–9, anterior end oblique, partly from anterior, partly from median cut surface. Figs. 10–16, anterior end on median side of piece; longitudinal axis at right angles to original longitudinal axis. Figs. 17–22, two partial or complete anterior ends on median side of piece; longitudinal axis at right angles to original longitudinal axis.

the tables are recorded here because they show no evidence of polarity corresponding to the original longitudinal axis. Their numbers are not large enough to alter essentially the results.

IV. Two heads more or less fused (Figs, 17–21), or in *P. maculata* occasionally two complete heads (Fig. 22), develop with their axes parallel or nearly parallel and at right angles, or nearly so, to the original longitudinal axis of the piece. These two heads develop in large part from the median cut surface but anterior and posterior cut surfaces which are usually brought almost into the same plane, as the median surface in consequence of contraction may also be included to a greater or less extent in the head-forming region (Figs. 17–22), so that it is often impossible to determine whether the heads originated from anterior, posterior, or median cut surfaces. These forms do not develop a new posterior end.

THE FREQUENCY OF THE DIFFERENT FORMS

Planaria dorotocephala.—Table I gives data for half-transverse pieces, i.e., pieces representing half the width of the body, of different lengths, and from different levels of the anterior zooid region. The first column of the table gives the lengths of the pieces, one-third, one-fourth, etc., as fractions of the length of the anterior zooid. The second column indicates the body-level, *A* being always the most anterior piece, immediately posterior to the head; *B* the next piece; etc. The third column gives numbers of pieces cut; in this series, 100 of each. The fourth column gives numbers of pieces surviving at time of examination. In the four columns under the general head "Group Percentages of Survivors" the frequencies of the four groups are given as approximate percentages of the number of survivors in each case, fractions of per cents being eliminated.

As the table shows, almost all of the forms reconstituted fall into Groups I (Figs. 3–6); in other words, the longitudinal axis of the reconstituted form corresponds in direction to the longitudinal axis of the original individual, but, averaging for all body-levels, a slight decrease in frequency of these forms appears with decrease in length of piece (one-third pieces, 99.7; one-fourth pieces, 97.5; one-fifth pieces, 95.2; one-sixth pieces, 92). The percentages of the forms in Group II with anterior ends developing in part from the median,

in part from the anterior cut surface and oblique to the original longitudinal axis (Figs. 7–9), increase with decrease in length of the pieces. The total for all levels is 1 per cent in one-third pieces, 10 per cent in one-fourth pieces, 18 per cent in one-fifth pieces, and 27 per cent in one-sixth pieces.

TABLE I

HALVES OF TRANSVERSE PIECES OF ANTERIOR ZOOID: *Planaria dorotocephala*, 16–18 MM. IN LENGTH

PIECES	LEVELS OF PIECES	NUMBERS OF PIECES	NUMBERS SURVIVING	GROUP PERCENTAGES OF SURVIVORS			
				I	II	III	IV
1/3	A	100	35	100	.....	.....	.....
	B	100	28	100	.....	.....	.....
	C	100	80	99	1	.....	.....
1/4	A	100	57	100	.....	.....	.....
	B	100	71	100	.....	.....	.....
	C	100	29	96	4	.....	.....
	D	100	82	94	6	.....	.....
1/5	A	100	85	99	1	.....	.....
	B	100	83	94	6	.....	.....
	C	100	36	88	9	3	.....
	D	100	69	97	1	1	1
	E	100	87	98	1	1	.....
1/6	A	100	72	93	1	6	.....
	B	100	68	87	10	3	1
	C	100	89	82	12	4	2
	D	100	75	95	1	4	.....
	E	100	86	97	2	1	.....
	F	100	87	99	1	.....	.....

Group III with a single anterior end on the median cut surface (Figs. 10–16) shows still lower frequencies: none in one-third and one-fourth pieces, 1 per cent in one-fifth pieces, and 3 per cent in one-sixth pieces. Forms of Group IV with two anterior ends (Figs. 17–22) do not occur in one-third and one-fourth pieces, and only rarely in one-fifth and one-sixth pieces. In general, the table shows that in the great majority of surviving half-transverse pieces the longitudinal axis corresponds in direction to the original longitudinal axis, that the frequencies of such forms decrease slightly, and that the frequencies of Groups II, III, and IV increase with decrease in length of piece. These data for half-transverse pieces which include a portion of one of the longitudinal nerve cords are

given for the purpose of comparison with the data in the following tables for pieces representing the region lateral to the longitudinal nerve cords.

Table II gives the frequencies for pieces of *P. dorotocephala* lateral to the nerve cords. It is similar in arrangement to Table I; but since it includes series from animals of different lengths and pieces both from the anterior zooid only and from the entire body, the first and second columns give the data concerning these points. Table II shows throughout a striking difference from Table I, in that Group III with anterior end on the median cut surface includes the great majority of the survivors. Group I is next in frequency, though much lower than Group III; and Groups II and IV are both in general still lower, although higher than Group I in certain lots. It is evident, then, that in these pieces from regions lateral to the longitudinal nerve cords most of the survivors develop heads on the median instead of on the anterior cut surface. In the first two sections of Table II data are given for the anterior zooid region of animals 20–22 mm. and 16–18 mm., respectively; and the percentage frequencies in these two sections show a very similar group distribution, as might be expected. The third section gives data for one-sixth pieces of the entire body-length of 20–22 mm. animals. The first three of these pieces *A–C* represent approximately one-third pieces of the anterior zooid, and the frequency distribution is in general very similar to that in the one-third pieces of the first two sections of the table. In the pieces *D*, *E*, and *F* of the third section, which represent approximately the posterior zooid region, the distribution is similar to that at other body-levels.

The fourth section of Table II includes one-third and one-fourth pieces of the anterior zooid region of small physiologically young animals 8–10 mm. in length. Here the frequencies of Group I are somewhat higher than, those of Group II and IV somewhat lower than, and those of Group III about the same as in pieces of the same relative length, i.e., one-third and one-fourth of the anterior zooid, from large animals. The pieces from the small animals are of course much smaller than the corresponding pieces from the large animals, but this difference in size has no marked effect upon the frequency distribution in the four groups.

TABLE II

PIECES FROM REGION LATERAL TO NERVE CORD: *Planaria dorotocephala*

Length of Animals (in Mm.)	Region of Body Sectioned	Pieces	Levels of Pieces	Numbers of Pieces	Numbers Surviving	Group Percentages of Survivors			
						I	II	III	IV
20–22	anterior zooid	1/3	A	80	44	18	14	66	3
			B	80	48	21	8	48	23
			C	80	53	15	9	55	21
		1/4	A	80	30	13	13	73	.....
			B	80	41	12	2	80	6
			C	80	52	6	2	83	10
			D	80	45	9	5	77	9
		1/5	A	80	34	9	3	89	.....
			B	80	35	20	3	77	.....
			C	80	38	8	13	74	5
			D	80	38	3	21	71	5
			E	80	39	5	3	80	13
		1/6	A	80	24	4	4	92	.....
			B	80	29	7	7	83	3
			C	80	37	3	3	95	.....
			D	80	27	4	.....	89	7
			E	80	29	3	.....	86	10
			F	80	32	13	9	63	16
16–18	anterior zooid	1/3	A	100	40	20	8	72	.....
			B	100	53	15	4	60	22
			C	100	62	10	.....	77	13
		1/4	A	100	32	13	6	81	.....
			B	100	26	4	7	73	15
			C	100	42	5	2	83	10
			D	100	58	5	3	85	7
		1/5	A	100	27	11	11	78	.....
			B	100	22	18	.....	77	5
			C	100	29	17	.....	83	.....
			D	100	39	8	5	87	.....
			E	100	40	10	5	85	.....
		1/6	A	100	39	5	5	90	.....
			B	100	33	.....	10	90	.....
			C	100	43	7	.....	93	.....
			D	100	40	8	3	90	.....
			E	100	54	11	2	85	2
			F	100	58	9	2	88	1
20–22	entire body	1/6	A	80	43	12	14	74	.....
			B	80	50	22	.....	68	10
			C	80	57	19	2	72	7
			D	80	49	18	2	76	4
			E	80	50	22	2	68	8
			F	80	52	15	.....	83	2

TABLE II—*Continued*

Length of Animals (in Mm.)	Region of Body Sectioned	Pieces	Levels of Pieces	Numbers of Pieces	Numbers Surviving	Group Percentages of Survivors			
						I	II	III	IV
8–10.....	anterior zooid	1/3	A	60	24	29	33	38	.....
			B	60	39	23	3	72	3
			C	60	38	13	5	79	3
		1/4	A	60	30	30	.....	70	.....
			B	60	30	13	7	80	.....
			C	60	37	30	3	62	5
			D	60	24	29	.....	71	.....

Table II shows no definite relation between frequency distribution and body-level. The percentages in pieces of the same length from different levels vary irregularly. Whether a relation between level and group distribution of forms might appear if the death-rate were lower cannot of course be determined. It may be noted, however, that survival does show a relation to body-level. In general the more posterior the level the larger the number of pieces surviving, but there is no reason to believe that the frequency distribution is essentially altered by differences in death-rate.

Table III gives the averages of the group percentages of Table II for pieces of each length from all levels of the body. Considering the averages of the four groups as given in columns I–IV of Table III, it appears that in general the shorter the piece, the higher the frequencies in Group III and the lower the frequencies in Group IV —that is, the shorter the piece, the more frequently does a single anterior end arise on the median cut surface and the less frequently do two anterior ends develop. In Group I the one-third pieces show the highest and the one-sixth pieces the lowest frequencies, but between the extremes irregularities occur. In Group II no great differences appear with difference in length of piece. In general it is evident from Table III that there is an increase in the frequency of single anterior ends on the median cut surface, with decrease in length of piece. This increase is 28 per cent from one-third to one-sixth pieces in the first section, 19.3 per cent in the second, 7.5 per cent in the fourth. Here, again, the question whether the high death-rate is a factor in determining these differences in frequency

must be raised, though it cannot be definitely answered. The last column of Table III gives the average numbers of survivors from all body-levels for each length of piece. In the first section there is a progressive decrease from 48 to 29.7, with decrease in length of piece. The second section shows a decrease from 51 to 31.4 from one-third to one-fifth pieces, but an increase to 44.5 in the one-sixth pieces. In the third section the one-sixth pieces of the entire bodies of large animals show a high average survival number, 50.2; but it

TABLE III

AVERAGE GROUP PERCENTAGES AND SURVIVAL NUMBERS OF LATERAL PIECES OF *Planaria dorotocephala* FOR ALL LEVELS
(From the data of Table II)

Length of Animals (in Mm.)	Region of Body Included	Length of Pieces	Groups				Average Survival Numbers
			I	II	III	IV	
20–22....	anterior zooid	1/3	18.0	7.0	56.0	15.7	48.0
		1/4	10.0	5.5	78.5	6.25	42.0
		1/5	9.0	8.6	78.0	4.6	36.3
		1/6	5.7	3.8	84.0	6.0	29.7
16–18....	anterior zooid	1/3	15.0	4.0	69.7	11.7	51.0
		1/4	6.75	4.5	80.0	8.0	39.5
		1/5	12.8	4.2	82.0	1.0	31.4
		1/6	6.7	3.7	89.0	0.5	44.5
20–22....	entire body	1/6	18.0	3.3	73.5	5.2	50.2
8–10....	anterior zooid	1/3	21.7	13.7	63.0	2.0	33.7
		1/4	25.5	2.5	70.5	1.25	30.3

must be remembered that these pieces correspond in length to one-third pieces of the first and second sections of Table III and show about the same survival number as those pieces. The fourth section shows again a decrease from one-third to one-fourth pieces of the anterior zooid of 8–10 mm. animals. With two exceptions, then, a decrease in average survival number occurs with decrease in length of piece. In other experiments with pieces of different lengths it is the general rule that in pieces below a certain length, the death-rate increases with decrease in length; but the actual numbers differ considerably in animals from different stocks and under different conditions. Undoubtedly, therefore, the decreases in survival num-

bers with decrease in length of piece are significant. It is improbable, however, that there is any relation between death or survival of a particular piece and the position in which the anterior end will arise if it survives, and in any case it is not to be expected that pieces which give rise to anterior ends on the median surface will

TABLE IV

PIECES FROM REGION LATERAL TO NERVE CORD: *Planaria maculata*

LENGTH OF ANIMALS (IN MM.)	REGION OF BODY SECTIONED	PIECES	LEVELS OF PIECES	NUMBERS OF PIECES	NUMBERS SURVIVING	GROUP PERCENTAGES OF SURVIVORS			
						I	II	III	IV
		1/3	A	60	19	11	6	73	10
			B	60	27	27	4	54	15
			C	60	35	26	3	61	6
		1/4	A	60	31	23	7	70	.....
			B	60	37	22	.....	65	13
			C	60	22	36	5	54	5
			D	60	37	17	.....	75	8
16–18.....	anterior zooid	1/5	A	60	20	20	.....	80	.....
			B	60	26	14	4	70	11
			C	60	13	38	.....	61	.....
			D	60	9	33	.....	56	11
			E	60	24	33	.....	62	4
		1/6	A	60	17	12	5	77	5
			B	60	25	8	4	88	.....
			C	60	24	12	.....	88	.....
			D	60	18	22	6	66	5
			E	60	14	14	.....	86	.....
			F	60	12	33	.....	66	.....
20–22.....	entire body	1/6	A	40	29	21	31	48	.....
			B	40	31	16	.....	84	.....
			C	40	32	22	.....	78	.....
			D	40	35	20	3	71	6
			E	40	18	6	.....	94	.....
			F	40	25	24	16	60	.....

survive more frequently as length of piece decreases. Since such forms become more frequent as length of piece decreases, the conclusion is justified that this increase in frequency is really correlated with decrease in length of piece.

Planaria maculata.—The data on this species are less extensive than those on *P. dorotocephala* because of the impossibility of obtaining sufficiently large numbers of animals. Table IV, which is

similar to Table II, gives the data obtained and shows, like Table II, the highest percentage frequencies in Group III, with Group I next but always much lower and only small numbers in Groups II and IV. Evidently in this species, as in *P. dorotocephala*, the great majority of these lateral pieces develop an anterior end on the median cut surface. Table IV also resembles Table II in showing no definite correlation between body-level and frequency distribution.

Table V gives the averages for all body-levels of the group percentages and survival numbers of pieces of different lengths as given in Table IV, and may be compared with Table III. It is evident

TABLE V

AVERAGE GROUP PERCENTAGES AND SURVIVAL NUMBERS OF LATERAL PIECES OF *Planaria maculata* FOR ALL LEVELS

(From the data of Table IV)

LENGTH OF ANIMALS (IN MM.)	REGION OF BODY INCLUDED	LENGTH OF PIECES	GROUPS				AVERAGE SURVIVAL NUMBERS
			I	II	III	IV	
16–18....	anterior zooid	1/3	21.3	4.3	63.0	10.3	27.0
		1/4	24.0	3.0	66.0	6.5	31.75
		1/5	27.6	0.8	66.0	5.2	18.4
		1/6	18.5	2.5	78.5	1.7	18.3
20–22....	entire body	1/6	18.2	8.3	72.5	1.0	28.3

that within the limits of length included, length of piece has less effect on the average frequency distribution in this species than in *P. dorotocephala*. In Groups I and II the differences are slight and irregular. In Group III there is no significant difference from one-third to one-fifth pieces, but an increase of 12.5 per cent from the one-fifth to the one-sixth pieces. Group IV, on the other hand, shows a progressive decrease from one-third to one-sixth pieces, that is, the shorter the piece, the less frequently do two anterior ends develop. This is the only group which shows a consistent progressive change in frequency throughout.

Comparing Table V with Table III, it appears that the averages of Group I are in general somewhat higher, and those of Group III somewhat lower, in *P. maculata* than in large individuals of *P. dorotocephala* (first three sections of Table III). In fact, they are

more like those of the 8–10 mm. individuals of *P. dorotocephala* (fourth section of Table III). Apparently, in the lateral pieces of large individuals of *P. maculata* the longitudinal axis of the form developed corresponds in direction with the original longitudinal axis more frequently than in the large individuals of *P. dorotocephala*.

DISCUSSION

The first point of interest is the very striking difference between the half-transverse pieces (Table I) and the pieces from regions lateral to the longitudinal nerve cords of *P. dorotocephala* (Tables II and III), as regards the position of the anterior end and the direction of the longitudinal axis of the reconstituted form. In the half-transverse pieces the original direction of the longitudinal axis persists in almost all of the pieces, while in the great majority of the lateral pieces the anterior end develops from the median cut surface so that the longitudinal axis of the reconstituted form is at right angles to the original axis. No data on half-transverse pieces of *P. maculata* were obtained, but the lateral pieces show high frequencies of change in direction of axis, and the other similarities in reconstitution of the two species afford ground for believing that half-transverse pieces would show frequencies very similar to those obtained in *P. dorotocephala*. The lateral pieces of *P. dorotocephala* show in general an increase in the frequency of change in direction of axis with decrease in length of piece. In *P. maculata* this change appears only in the shorter pieces. Neither species shows any relation between such change and body-level.

As regards the possible factor or factors concerned in determining the difference in position of the anterior end in half-transverse and lateral pieces, it is evident that this difference depends on some difference in the piece which is not merely incidental or occasional, but very generally present and effective. Such differences are, on the one hand, those immediately associated with section, such as differences in size and form of piece and in relative areas of anterior and median cut surfaces, and on the other hand, those physiological differences dependent on region of the body or on particular organs or tissues included in the piece or on relation of the piece to the general body gradients.

In general the lateral pieces are less than half the volume of the half-transverse pieces of the same length, since the dorsoventral thickness of the lateral is less than that of the median regions. On the other hand, one-third lateral pieces are almost as large as one-sixth half-transverse pieces, but the difference in frequency of anterior ends on the anterior cut surface between such pieces is about 80 per cent. Moreover, the lateral one-third and one-fourth pieces of 8–10 mm. animals, which are very much smaller than those of 20–22 mm. animals, show almost as high a frequency of change in direction of axis as the larger pieces. Evidently size and volume of the pieces are not important factors in the result.

Form of piece and relative areas of cut surfaces are of course different in half-transverse and lateral pieces. In lateral pieces the width is less and the relative area of median, as compared with anterior, cut surface is greater than in half-transverse pieces of the same length; but the form of the one-sixth lateral pieces approaches that of the one-third half-transverse pieces, while the relative area of median cut surface decreases with decrease in length of piece. The frequency of anterior ends on the median cut surface is very much higher in the narrower lateral pieces with greater relative area of median cut surface than in half-transverse pieces, but it also increases with decrease in length of piece, that is, with increase in relative width of piece and decrease in relative area of median cut surface. In short, the data indicate that form and volume of piece and relative areas of anterior and median cut surfaces have little if anything to do with position of the anterior end.

A relation between length of piece and position of anterior end does appear in the lateral pieces and to a slight extent in the half-transverse pieces. The shorter the piece, the more frequently does the anterior end develop on the median surface; but this fact alone throws no light on the nature of the relation concerned.

As regards organs and tissues present, the half-transverse pieces include parts of the longitudinal, as well as lateral, branches of the alimentary tract and a part of one longitudinal nerve cord, while the lateral pieces contain only lateral branches of the alimentary tract and lateral branches of the nerve cords. These lateral nerve branches, however, contain cells as well as a fibrillar tract and re-

semble the longitudinal cords except in size. They, or at least their more central portions, are undoubtedly to be regarded as belonging to the central nervous system, a possibility which seems to have been ignored by Morgan (1898, 1903) and Bardeen (1903) in their attempts to determine whether the development of a head depends on the presence of the central nervous system.

It seems evident from the positions of the anterior ends in the half-transverse and lateral pieces that the alimentary tract is not an important factor in localizing the anterior end, but it is true that in pieces of *Planaria* heads very often develop on the cut surface which contains the central end of the part of the nervous system present. In transverse and half-transverse pieces this is the anterior end; in lateral pieces, the median surface. But in short transverse pieces heads sometimes develop from the posterior cut surface, and in half-transverse pieces sometimes in part from both anterior and median cut surfaces and sometimes from the median surface. Evidently the central cut end of a part of the nervous system is not the only factor concerned in their localization.

The lateral branches of the nerve cords are so numerous that even the shortest pieces used in these experiments contain more than one. Contraction of the cut surfaces in these pieces brings the central ends of these branches more or less together on the median surface, and we find a high frequency of anterior ends on this surface. In a considerable number of cases, however, the head arises on the anterior cut surface or obliquely from both anterior and median surfaces; and in some cases two heads develop, both in large part from the median surface. Some of the shorter pieces show no outgrowth of new tissue on any cut surface and remain apparently apolar.

With respect to the question of the relation between the central nervous system in the piece and the development of cephalic ganglia in the new head, it has been stated by various investigators (Flexner, 1898; Schultz, 1902; Keiller, 1910; Lang, 1912; Olmsted, 1918) that the cephalic ganglia arise, or may arise, in the new tissue independently of the portions of the nervous system present in the old tissue. Stevens (1901) states that the nervous tissue extends from the old into the new tissue and that the new ganglia develop from embryonic

cells which group themselves about this nervous tissue. Lehnert (1891) and Bardeen (1903) regard the old portions of the nervous system present as directly concerned in the formation of the new ganglia.

The fact that a new head develops directly from a cut surface at any postcephalic body-level in the complete absence of the parts normally present between that level and the head constitutes strong evidence for the view that the head as a definite organ complex is not determined by more posterior levels of the body, though its development may be inhibited by them. In anterior reconstitution, as in embryonic development, the head develops first, and the other parts normally present between it and the level from which the piece was taken are reorganized in sequence posterior to it, as far as size of the piece permits. Apparently the piece determines only where or whether the activation of cells which give rise to the head shall occur. As regards relations to the rest of the piece, the reconstitution of a head appears to be primarily a self-differentiation. Every level of the body will give rise to a head when it is not subordinated to other levels. This conception of head reconstitution has developed from various lines of experimental evidence, and there is at present no evidence in conflict with it. If it is correct, the cephalic ganglia undoubtedly arise independently of the parts of the old nervous system present in the piece and there is no ground for regarding a part of the central nervous system as a necessary factor in the localization of a new head. It is still, however, an interesting question whether the nervous system plays any part as nervous system in localizing a new head, or whether its central cut end is merely a landmark for other physiological factors.

As already noted, heads do not always arise in the same relation to the central nervous system in the piece. They may fail to develop at the central cut end, and they may develop elsewhere. In transverse pieces the frequency of head formation decreases with decreasing length of piece (Child, 1911*a;* Sivickis, 1923). This is due to an effect of some sort from the posterior cut surface, for if the posterior cut is made a few hours later than the anterior the head is not inhibited even in pieces so short that all are headless when the posterior and anterior cuts are made at the same time (Child,

1914). If, on the other hand, the posterior cut is made first, it will inhibit head development until it closes. If it is allowed to close before the anterior cut is made, head development is not inhibited, even in pieces which would be headless if both posterior and anterior cuts were made at the same time (Child, unpublished). In pieces below a certain length and of the same length from different body-levels the frequencies of head formation show a gradation according to body-level and can be altered by various external physical and chemical agents (Buchanan, 1922; Child, 1911*a*, 1916, 1920, 1921; Sivickis, 1923). These experiments have been repeatedly performed with the same results by students in the laboratory, and, together with other data, indicate that conditions at the cut surface on which the head develops are not the only factors in determining whether or not a head shall develop. Conditions in other parts of the piece can at least inhibit head formation.

Many lines of evidence which have been reviewed elsewhere (Child, 1928) indicate or demonstrate that graded differentials in physiological condition, apparently primarily quantitative in character, are characteristic features of physiological axes, at least in the earlier developmental stages, and more or less throughout life in many of the simpler organisms. Usually, however, the simple axial gradients of earlier stages undergo progressive complication and modification as the various organs develop, and may persist only in certain parts of the body or may be completely obliterated. In the case of *Planaria* the evidence indicates the presence in the body-wall and apparently in the parenchyma of a longitudinal gradient from the head to the region at which fission occurs, or in species which do not undergo fission, to the growing region at the extreme posterior end and a gradient extending laterally and dorsally from a median ventral zone. In the gradients anterior and median ventral regions represent the highest levels of physiological activity. The differentiation of the central nervous system apparently takes place down these gradients, that is, from anterior to posterior and from the median zone laterally so that in its distribution it becomes an expression of the gradients.

The evidence indicates further that in reconstitution heads develop from the most active cut surface of an isolated piece. Under

ordinary conditions this surface is determined by the highest gradient level in the piece; e.g., in transverse pieces it is usually the anterior surface. It is possible, however, to inhibit this region and to activate some other and so alter the localization of the head experimentally. According to this view, the anterior cut surface represents the highest level in the anteroposterior gradient in transverse pieces and usually in half-transverse pieces, and the median surface usually the highest level in lateral pieces. These surfaces contain the central cut ends of the parts of the nervous system present, and the data show that they usually give rise to the anterior end. Considering only these usual cases, we might conclude that the nervous system was the essential localizing factor; but the cases in which heads develop in other positions show that this cannot be true. In both half-transverse and lateral pieces anterior ends develop obliquely, in part from the anterior, in part from the median cut surface; also a few of the shorter half-transverse pieces develop an anterior end, or occasionally more than one, on the median surface. In a considerable number of the lateral pieces the anterior end develops on the anterior cut surface.

In the lateral, and occasionally in the half-transverse, pieces conditions at the anterior and median cut surfaces are apparently so nearly balanced that in some cases the one, in some cases the other, becomes the determining factor; or both may work together in localizing the anterior end obliquely to the original axis as a resultant of conditions on both. Whatever the nature of these conditions, it is evident that they are similar in nature on both cut surfaces, for the localizations of the anterior ends constitute a continuous series from anterior to median surface and often involve both in part. Also, localization on the median surface is more frequent in the shorter than in the longer pieces, and much more frequent in the lateral than in the half-transverse pieces. Apparently such localization can be accounted for only in terms of two physiological differentials similar in character but at right angles to each other. Of these differentials, either may become the determining factor in localization, or the two may work together and localize the anterior end as a resultant of the combined action. In short, it seems evident that the two gradients, anteroposterior and mediolateral, must be

regarded as the primary factors determining the localization of the anterior end.

This interpretation does not at all exclude the possibility that the nervous system plays some part in localizing anterior ends. In the half-transverse pieces, the central cut end of the nerve cord is anterior, and the new anterior end arises on the anterior cut surface in almost all cases. In the lateral pieces, the central ends of the lateral nerve branches present in the piece are more or less approximated on the median surface by the contraction, and the anterior end usually develops there. If the nervous system is a factor in such localization, the central cut ends of the lateral branches are evidently less effective than the central cut end of a longitudinal nerve cord, for we find that anterior ends develop much more frequently on the anterior surface in lateral pieces than on the median surface in half-transverse pieces. Apparently, also, the cells about or between the central ends of the nerve cords or branches present are activated and grow more rapidly than others, for the outgrowth of new tissue on a cut surface is more rapid in the median ventral zone than elsewhere. It may be that the nervous system plays a part in maintaining this high reactivity in these cells. But even if this is true, there is no evidence that such action is in any way specific in determining an anterior end, for anterior ends may be localized elsewhere provided the cells concerned are sufficiently activated. Apparently a head may develop from any cut surface which exposes parenchyma cells if the cells can be made sufficiently active in relation to other parts. The nervous system may assist in such activation; but if that is the case, the fact that the anterior end usually arises at the central rather than the peripheral cut end of the part of the nervous system present indicates that a physiological differential between central and peripheral surfaces exists. In very short pieces this differential may be so slight that the two surfaces are essentially alike and develop like parts or merely heal. In other words, the nervous system, as a possible factor in the localization of an anterior end, is itself an expression of the gradients, and any other factor which determines the necessary differential may determine localization of an anterior end.

To sum up, the central end of that part of the nervous system

present in the piece may be merely an indicator of a certain level of physiological condition in the cells about it, or it may be a factor in maintaining that level. Whichever the case, there is no evidence of any specific effect of the nervous system in localizing a head. Any effect which does exist must be regarded as an expression of the gradient along the axis concerned. If this is true, the cut surface characterized by the presence of the central cut end of a part of the nervous system is merely a preferential locus for head formation, and any other cut surface on which the cells are sufficiently activated by conditions of any sort may give rise to a head.

The point is to be emphasized that, according to this conception, the central nervous system, if it is a factor, is merely a localizing, not a determining, factor in head formation. The recent work of Guyénot and Schotté (Guyénot, 1927; Guyénot et Schotté, 1926; Schotté, 1926*a*, *b*) shows that peripheral nerves may function as activating and localizing factors without determining the character of the part which develops. In so far as the central nervous system influences the development of the anterior end in *Planaria*, it must be regarded as an activating factor concerned in localization, and not as a determining factor.

Whether the nervous system plays any part in determining the presence of two partial or complete heads on the median surface (Figs. 17–22) is unknown. In the half-transverse pieces the presence of cut ends of two transverse commissures may perhaps determine two regions of rapid activation and growth of cells about them and so localize two heads. In the lateral pieces the central cut ends of the lateral nerve branches may sometimes be brought into two groups by the contraction, or two relatively large branches at some distance from each other may determine more or less separate regions of growth. The data show that frequency of two heads decreases with decrease in length of piece, i.e., the shorter the median cut surface, the less likely it is to give rise to two heads.

Olmsted's conclusion (1918) that the development of a head on a median cut surface does not involve a fundamental change in polarity appears not to be in accord with the facts. When the head develops on the median surface, the direction of the polar axis is altered by 90° and is in the direction of the mediolateral instead of

the longitudinal differential. Locomotion takes place in the direction of the new axis, and this means a change in the direction of ciliary action. This change in polarity, however, does not represent a new axis impressed on the piece by external factors at the time of, or after, section, but evidently results primarily from conditions present in the region of the body concerned while it was still a part of the intact animal, although these conditions may be influenced and more or less altered by length of piece and other factors, so that the new anterior ends do not always arise on the median surface.

SUMMARY

1. Half-transverse one-third, one-fourth, one-fifth, and one-sixth pieces of the anterior zooid of *Planaria dorotocephala* show frequencies approaching 100 per cent of the survivors of anterior ends developing from the anterior cut surface.

2. One-third, one-fourth, one-fifth, and one-sixth pieces of the anterior zooid of *Planaria dorotocephala* from regions lateral to the longitudinal nerve cords show high frequencies of anterior ends developing from the median cut surface.

3. Much lower frequencies of anterior ends developing obliquely in part from both anterior and median cut surfaces and of two partial or complete heads, largely from the median cut surface, occur in the lateral and the shorter half-transverse pieces.

4. In the lateral pieces the frequencies of anterior ends on the median cut surface show no relation to level of body, but increase with decrease in length of piece. The frequencies of forms with oblique anterior ends show no definite relation to length of piece. Forms with two heads decrease in frequency with decrease in length of piece.

5. One-third, one-fourth, one-fifth, and one-sixth pieces of the anterior zooid of *Planaria maculata* from regions lateral to the nerve cords show frequencies of position of new anterior ends very similar to those in *Planaria dorotocephala*.

6. It is shown that differences in size and form of piece or in relative areas of cut surfaces cannot account for the differences in position of anterior ends, but that when parts of the central nervous system are present anterior ends usually, but not always, develop

from the cells about the central cut ends of these parts. In half-transverse pieces these are on the anterior cut surface; in lateral pieces, on the median surface.

7. The problem of the factors concerned in localization of anterior ends in pieces is discussed and the conclusion drawn that the nervous system is not an essential factor, though it may play a part in localization through its influence on the physiological condition of the cells about it. The nervous system is regarded as an expression of the physiological gradients in the body and these, or new gradients determined by the conditions of experiment, are considered to be the only factors which can account for the observed facts of such localization.

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THE EFFECT OF INTRACELLULAR CYANIDE ON CLEAVAGE OF ARBACIA EGGS

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IT IS well known that weak acids penetrate living cells with little, if any, resistance. It has also been shown (Jacobs, 1920; Beerman, 1924; Bodine, 1924; Osterhout and Dorcas, 1925; Brinley, 1927, 1928, 1929) that certain weak acids, namely, carbon dioxide, hydrogen sulphide, and hydrogen cyanide, enter living cells in the form of undissociated molecules. Certain authors (Jacobs, Bodine, Beerman) have suggested that the physiological effects of these acids may be due to their intracellular ionization. On the other hand, it has been shown by micro-injection experiments (Reznikoff and Chambers, 1927; Brinley, 1928, 1929) that solutions of carbon dioxide, hydrogen sulphide, and hydrogen cyanide do not kill *Amoeba* when injected into the cell, but that the organisms die very quickly when immersed in the solutions. These latter reports seem to indicate that the toxic action of these weak acids is due to some effect on the plasma membrane.

It is equally well known that these weak acids, especially hydrogen cyanide, in dilute solutions act in general as narcotics. Overton (1901), Traube (1913), Lillie (1916), and others contend that anesthetics effect the cell by virtue of their action on the plasma membrane. On the other hand, Heilbrunn (1920) maintains that the action of anesthetics may be explained in terms of viscosity changes of the internal protoplasm.

Since hydrogen cyanide is an extremely weak acid and behaves in many respects as an anesthetic, it was thought desirable to determine whether the effect of cyanide is due to its action on the internal protoplasm or to its effect on the plasma membrane. Owing to the

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fact that considerable work has been done on the effect of cyanide and anesthetics on the cleavage processes of *Arbacia* eggs, it was deemed advisable to study the effect of intracellular and extracellular cyanide on the time of first cleavage of these eggs.

METHOD AND RESULTS

Aqueous solutions of potassium cyanide in distilled water were used in the following experiments. Potassium cyanide in aqueous solutions ionizes in the following manner:

$$KCN + H_2O \rightleftharpoons KOH + HCN$$

$$KOH \rightleftharpoons K + OH$$

The toxicity of KCN is probably due to the formation of molecular HCN (Brinley, 1927), although the K and OH ions may modify this toxic effect. Various concentrations of KCN, ranging from M/100 to M/1,000 were used; but as the effect was the same for all concentrations, this report deals only with the experiments conducted with M/100 KCN solution. The eggs from ripe females were removed in the usual manner and fertilized in finger bowls. At various times after the formation of the fertilization membrane a number of eggs (containing fertilization membranes) were injected with M/100 solution of KCN by means of Chamber's micromanipulator. The eggs were placed in a hanging drop in the moist chamber, and those selected for injection were drawn to the edge of the drop where the surface tension of the water held the eggs tightly against the cover glass. Large dosages, equal to about one-fourth the volume of the cell, were injected into the cell as rapidly as possible, and the eggs were then returned to the deeper portion of the drop. Great care was exercised in injecting to be sure that the cyanide solution was actually placed in the cell and not simply into an invagination of the cell membrane. In many cases where large dosages were injected a portion of the egg protruded, forming a bud on the side of the cell. The protoplasm flowed away from the point of the pipette and the solution was seen to enter the cell. The cyanide rapidly diffused through the cell and mixed with contents of the egg. Experiments conducted by injecting distilled water containing NaOH in sufficient quantities to obtain the same pH as the KCN

solution showed that the alkalinity of the solution was not an important factor in the behavior of the intracellular cyanide. Injections of minute amounts of sea water into the cell resulted in a local cytolysis which rapidly spread from the point of entrance over the entire egg. The reddish pigment in the eggs turned light orange, and later a reddish purple as cytolysis proceeds.

During the course of the experiment sixty-four fertilized *Arbacia* eggs were successfully injected with large dosages of M/100 KCN solution, made up in distilled water. The time of first cleavage of the injected eggs and the time of first cleavage of control eggs in the same hanging drop was determined. The mean of the time of first cleavage of the injected eggs was 63.5 ± 0.49 minutes, while the mean of the control eggs was 59 ± 0.33 minutes. The difference in time of cleavage between the injected and control eggs was 4.5 ± 0.59 minutes, which may be considered as a significant difference as it is over seven times the probable error. However, it was also determined that cleavage was delayed to the same extent whether the eggs were subjected to injury by microdissection needles, injected with distilled water, or injected with cyanide solution. A number of eggs were injected at various times after the formation of the fertilization membrane to determine if the time of injection after fertilization affected the time of cleavage. Cells injected just before cleavage continued to divide, and cleavage was not delayed to any significant degree above the time of division of the controls. If injections were made near the completion of the cleavage furrow, the furrow disappeared and the cell assumed its original form, but immediately a new furrow was formed and cell division continued. This reaction also occurred when the eggs were injected with distilled water or severely pricked with a micropipette. Therefore, the time of injection between the formation of the fertilization membrane and just prior to cleavage does not seem to have any effect on the time of first cleavage. An attempt was made to inject one cell of the two-celled stage, but this could not be satisfactorily accomplished without some disturbance to both cells, which resulted in a delay of the second cleavage.

It is apparent, therefore, that the delay of the first cleavage of the injected eggs is not due to the anesthetic action of the cyanide

but to the disturbance produced by insertion of the pipette. In every experiment it was observed that the control eggs in the hanging drop divided at a much slower rate than those kept in finger bowls. In many cases this delay amounted to 10–15 minutes. This indicates that the conditions existing in the hanging drop are somewhat different from those in the finger bowls. However, the injected and control eggs were under identical conditions in the hanging drop.

Blumenthal (1928) reported that the first cleavage of *Arbacia* eggs is delayed when they are placed temporarily in KCN solution. The concentration of cyanide varied from M/100 to M/1,000, and the eggs were exposed for different periods of time. The length of delay depends upon the concentration of cyanide but is independent of the length of exposure. Eggs immersed temporarily in other anesthetics, such as isopropyl alcohol, ethyl acetate, and *n*-propyl alcohol, cleaved at the same time as did the controls. From these results Blumenthal concluded that KCN effects the internal protoplasm, thus differing from the other anesthetics which act on the plasma membrane. A portion of Blumenthal's work was repeated, using short exposures. At 10-minute intervals after fertilization the eggs were placed for 30 seconds in M/100 KCN solution. At the end of that time the eggs were removed by means of capillary pipettes, placed, and allowed to develop in finger bowls containing 200 cc. of sea water. It was found that first cleavage was delayed for a period of from 10–15 minutes, a confirmation of the results obtained by Blumenthal.

From the results of the preceding experiments it is evident that micro-injections of potassium cyanide do not delay cleavage of *Arbacia* eggs, whereas cell division is delayed when the eggs are immersed in cyanide for 30 seconds or longer. There seem to be several possible explanations for the foregoing facts: (1) cyanide effects the plasma membrane and not the internal protoplasm; (2) the intracellular cyanide is rapidly oxidized or unites with some cell constituent producing a non-toxic compound; (3) the cyanide quickly diffuses out of the cell after injection. Perhaps the simplest theory, and the only one to be discussed in this paper, is that the toxic or anesthetic action of cyanide is due to its effect on the plasma

membrane and not on the internal protoplasm. The fact that cyanide does have some effect on the cell membrane, especially on the permeability, has been reported by various investigators. Lillie (1918) states that the increase in permeability of *Arbacia* eggs following fertilization is reversibly retarded by KCN in concentrations of M/100 to M/400. Blumenthal (1927) suggested that KCN produces a decrease in permeability of the cell membrane of *Arbacia* eggs to water, while HCN increases the permeability. An additional reason for believing that cyanide acts on the surface membrane is that some internal development, such as mitotic figures, proceeds to a certain extent while the eggs are immersed in the cyanide solutions (Blumenthal, 1929). Heilbrunn (1920) studied this continuation of development and maintains that the effect of cyanide can be explained in terms of viscosity changes of the internal protoplasm. Heilbrunn states that in the normally developing egg there is a gradual increase in viscosity of the protoplasm to a certain stage where the process becomes reversible and the viscosity is decreased. In the presence of cyanide, Heilbrunn states, the gelation of the protoplasm is intensified and the normal decrease in viscosity which occurs during the mitotic process is rendered reversible and further development cannot take place. Thus the early stages of development proceed as far as the spindle and then stop. The particular stage of mitosis which is associated with a decrease in viscosity cannot take place in cyanide (Heilbrunn). However, it may be possible to explain this reaction in some other terms, such as a change in the colloidal nature of the plasma membrane which may accompany the internal changes. Heilbrunn did not report on the changes in the physical nature of the membrane. As the membrane is only modified protoplasm, may not the nature (viscosity) of the membrane change as the internal protoplasm changes? If the effect of cyanide is a surface one, it may be assumed that internal development would continue to a certain stage where, for further development, the process depends upon a change in the membrane which is prevented from occurring in cyanide. Lyon (1902) has shown that the resistance of *Arbacia* eggs to KCN depends on the time after fertilization that the eggs are immersed. The resistance of the eggs diminishes for the first 10–15 minutes after fertilization; then the

resistance increases until the time of first cleavage. This seems to be contradictory to Heilbrunn's conclusions. Lillie (1918), in a comment on Lyon's paper, states that the susceptibility to cyanide following fertilization is probably directly connected with changes in the properties of the plasma membrane.

A possible reaction between the cyanide and the plasma membrane may be explained by a combination of the cyanide with some constituent of the membrane. Warburg (1923 and other papers) states that cyanide inactivates the iron-containing substance (haemin) in the surface layers. It may also be possible that the cyanide changes the colloidal nature of the membrane.

SUMMARY

The results of experiments on immersion of *Arbacia* eggs in sea water containing M/100 KCN and by micro-injection of KCN solution into the eggs indicate that the anesthetic action of cyanide is on the plasma membrane. It is also suggested that the effect on the membrane may be due to the combination of the cyanide with some constituent of the membrane or a change in the colloidal nature of the membrane.

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METAMORPHOSIS OF THE AXOLOTL FOLLOWING LUNG EXTIRPATION

(Three figures)

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VARIOUS theories have been advanced to account for the non-metamorphosis of certain of the perennibranchiate amphibians such as *Necturus*, the assumption being that these forms are permanent larvae which have lost the ability to transform.

The most recent of these theories is that advanced by Figge (1929) in a paper entitled "A Morphological Explanation for the Failure of *Necturus* to Metamorphose." Figge points out that axolotls, a metamorphosing form, differ anatomically from *Necturus* in that the latter lacks the sixth aortic or pulmonary arch, the spiral valve, and septa in the truncus arteriosus. He expresses the opinion that "the physiological importance of these structures in a pulmonary respiratory system, and therefore in the adult or metamorphosed amphibians, suggests that the absence of these structures is responsible for the failure of Necturus to metamorphose (i.e., lose its gills and breathe by means of lungs)."

Figge tested his idea that the sixth arch is necessary for metamorphosis in those forms that do transform. He ligated the arch and vessels on each side in axolotls and found that animals so treated failed to metamorphose when treated with metamorphosing substances, e.g., thyroid; whereas the unoperated control animals did transform when treated with similar substances. Figge's complete paper has not yet appeared in print, and at the present writing only the abstract is available. However, his results and conclusions are clearly stated.

In view of the fact that there are certain lungless salamanders which metamorphose, namely, *Eurycea*, it was thought hardly credible that pulmonary respiration should play such a great part in the metamorphosis of urodeles, and axolotls in particular. Consequently the experiment was attempted of metamorphosing axo-

lotls whose lungs had previously been extirpated. Although not exactly the same as the ligation of the sixth aortic arch performed by Figge, the complete removal of the lungs was undertaken with the same purpose in mind, namely, to determine the effect of pulmonary respiration on metamorphosis, it being understood that complete removal of the lungs does away with the possibility of pulmonary respiration as effectively, to say the least, as ligations about the pulmonary arteries.

The writer wishes to express his indebtedness to Dr. W. W. Swingle, who suggested the problem, for his aid and suggestions.

The axolotls used were of the Colorado type received in Princeton January 3 through the General Biological Supply House, of Chicago. The animals averaged 135 mm. in length. Throughout the experi-

FIG. 1.—Location of incision for lung extirpation.

ment the controls as well as the experimental animals were kept in tanks through which compressed air was bubbled constantly. During the experiment the animals were fed once a week with raw liver chopped up very finely, and were kept well nourished.

The complete extirpation of the lungs was quite a simple operation, since no bleeding was involved. The animals were first anesthetized by means of chloretone (Park, Davis & Company, Detroit), a few crystals of which were dissolved in a finger bowl in which the animals were placed for about 15 minutes preceding the operation. It was found advisable not to completely anesthetize the animals since with the removal of the lungs the chances for coming out from under the anesthetic were reduced considerably. When rendered almost insensible, the animals were laid on a damp towel. A 6–8 mm. anterior-posterior incision was made just posterior and slightly dorsal to the fore limb (see Fig. 1). By reaching dorsally, it was easy to secure the lung, a reddish cylindrical organ 2–3 mm. in

diameter and about 20 mm. long, with the two blood-vessels. The free end of the lung was pulled out through the incision and cleared away anteriorly to the root, about which a very tight ligature was made to prevent a hemorrhage and possible regeneration. The lung was then cut off at the root as close to the ligature as possible, and the incision in the body wall was closed up with two or three stitches. Both lungs were removed at one operation; however, a separate incision was necessary for each lung. After the operation the animals were placed in a tank of fresh water through which a constant stream of air was bubbled. The water was kept well aërated at all times.

After removal of the lungs the axolotls showed a marked tendency to swim to the surface of the water for air at short intervals. Evidently the loss of pulmonary respiration was keenly felt. After a few days this tendency of rising to the surface for air stopped. No sooner was a mouthful of air taken in than it was expelled immediately on closure of the mouth.

When the animals had completely recovered from the effects of the operation, they were fed, by means of a pipette, some desiccated thyroid glands (Parke, Davis & Company). One feeding proved enough in every case to bring about metamorphosis. Quantitative feeding was deemed unnecessary since metamorphosis only was required.

The lungs were extirpated from two axolotls on January 9. One failed to come out from under the chloretone. The other recovered fully from the operation and was fed thyroid on January 16. By January 29 complete metamorphosis had taken place (Fig. 2: *a*, control; *b*, experimental animal). The animal, however, showed no tendency toward leaving the water. The tank was tilted so that the animal could crawl up the inclined surface to the air. Metamorphosis was normal except for the fact that the animal did not leave the water. The stages of metamorphosis were as follows: absorption of dorsal and caudal fins leaving a slight groove along the mid-dorsal line of the back, complete absorption of the external branchiae or gills preceded by the closure of the orifices on either side of the neck, protrusion of the eyes, shedding of the thin transparent epidermis several times, and development of the terrestrial

or adult type of pigmentation. On February 4 this animal was completely anesthetized with chloretone and photographed. It was then opened for examination. On the one side there was no trace of lung tissue. On the other side there was found a minute trace of lung tissue from which the blood supply was effectively cut off by the ligature at the root of the lung. The extremely small area of tissue was degenerate and non-functional.

On January 16 both lungs were extirpated from each of four axolotls. All of these survived the operation and after recovery were fed thyroid on January 24. One of these died on January 28 before showing any signs of metamorphosis.

A second animal of those operated on January 16 died on February 3. Metamor-

FIG. 2.—*a*, Control, not metamorphosed, having received no thyroid. *b*, Fully metamorphosed lungless axolotl.

FIG. 3.—*a*, Control, thyroid-fed and fully metamorphosed. *b*, Fully metamorphosed lungless axolotl. These two animals were preserved in formalin before photograph was taken.

phosis was complete except for a slight trace of external gills which appeared as round tubercles, although the gill filaments were completely resorbed. The orifices on either side of the neck had closed over. This animal was found dead on the dry end of the inclined tank. It probably crawled out into the air. On post mortem examination not a trace of lung tissue could be found.

A third animal of this group died on February 10. This animal, like the preceding one, tried to crawl out of the water; but after loss of the first animal the tank was filled, so that at the shallow end there was still a little water, just sufficient to keep the animal moist. However, on the morning of February 10 the water was found a little low and the animal dead, having crawled out into the air. In this animal metamorphosis was complete except for the presence of very small tubercles or remains of external gills. On examination, not a trace of lung tissue was found in this metamorphosed animal. This animal was the only one to show lack of complete co-ordination of the forelimbs. Probably the muscles were more seriously injured during the operation, since the wounded area extended in some cases into the pectoral region.

The fourth and last animal of those operated on January 16 exhibited no tendency toward leaving the water, although completely metamorphosed by February 9. On February 11 he was anesthetized and photographed, and recovered completely from the anesthetic. At the present writing this animal is still alive and normal in every respect—a typical metamorphosed *Amblystoma tigrinum* (Fig. 3: *a*, control, *b* experimental animal).

Two types of controls were used. One unoperated animal was fed thyroid on January 9 and 13. Metamorphosis was complete on January 16 and death occurred on January 26 (Fig 3*a*). To all external appearances the operated and metamorphosed animals were exact duplicates of this metamorphosed control. Note that the time required for metamorphosis was approximately the same for the operated animals and this unoperated control despite the fact that the control was twice fed thyroid. This time relationship for metamorphosis is not conclusive, since the feeding was not quantitative.

The other controls consisted of four unoperated and unmeta-

morphosed animals which received no thyroid. These animals have not shown any indications of metamorphosis though it is probable that they will transform spontaneously later on, since the Colorado axolotl is known to metamorphose spontaneously.

In view of the fact that the foregoing axolotls metamorphosed normally into adult *Amblystoma tigrinum* when fed thyroid, despite the total absence of lungs, the conclusion may be drawn that pulmonary respiration is unnecessary for metamorphosis of these animals. Since Figge's experiment of ligating the sixth arch is essentially the same in that it cuts off pulmonary respiration, his conclusion is evidently erroneous that "the sixth arch is necessary for metamorphosis," since axolotls have been made to metamorphose completely without lungs.

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FETAL AND EARLY POSTNATAL RESPONSES OF RAT GONADS TO PITUITARY INJECTIONS

(Eight figures)

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IT HAS long been known that the transplantation of the pituitary gland causes marked responses in Amphibia. These responses consist in acceleration of growth, hastened metamorphosis, and pigmentary changes (Allen, 1920; Swingle, 1923; Uhlenhuth, 1923; and others).

Smith and Engle (1927) have shown that daily transplantation of portions of the pars anterior of the mammalian pituitary gland into young rats causes striking responses in the genital system. These responses they attribute to the influence of the grafts upon the gonads, which in turn modify the entire genital tract by reason of their enhanced secretions.

The purpose of the present investigation was the determination of the earliest stage of development at which the organism is capable of direct response to the pituitary secretions. The effect of such secretions has been described by Smith and Engle, as noted above. We are not here, however, directly concerned with the effect, as such, but rather with the time at which the response occurs. Accelerated differentiation in the gonad has been used solely as an indicator of the organism's capability of response to the pituitary hormone. The writer wishes to make this distinction quite clear.

The present problem thus resolved itself into the introduction of pituitary substance into fetal, newborn, and young albino rats, with subsequent histological examination of the gonads at appropriate ages. Histological evidence of precocious maturity in the gonad was considered indicative of response to pituitary secretion at that age.

The method of extraction of the pituitary substance seems to be an important factor in experiments of this type, since differing results have been reported by various workers using identical methods

of preparation of the gland. Klinger (1919) reported that the injection of pituitary substance in saline solution produced no effect. Evans and Long (1921, 1922) state that increased weight and skeletal growth were the sole results of such treatment.

In order to observe the earliest effect of the pituitary hormone upon the young mammal, a method of injecting pituitary substance into the fetus was essential. Nicholas (1925) has devised a method for operating upon the fetus of the albino rat which has been eminently successful. A modification of this technique has beem employed by the author (Corey, 1928). Boucek (1928) employed a similar method of fetal injection in work upon placental permeability.

In a preliminary paper (Corey, 1928) experiments similar to the following were reported. Subsequently a more extensive study was made, and the complete data have been brought together in the present paper.

The writer here makes grateful acknowledgment to Professor J. S. Nicholas, of Yale University, for his many invaluable suggestions and unfailing interest in the work.

MATERIALS AND METHODS

Three qualities are necessary in the type of animal selected for fetal experimentation. The animal should be highly resistant to infection, since infection of the mother entails the loss of an entire litter. The animal must be able to withstand the operative shock, and must not abort following the manipulation of the viscera incident to operation. The fetus of the form selected must be of convenient size in order that its parts may be readily made out through the uterine wall, and injection effected in the correct location. The albino rat was selected as the subject for these experiments, since it possesses these essential qualities to a very marked degree.

Laparotomy and the subsequent injection of the fetuses was performed as follows: The pregnant animal was anesthetized with ether in a clean glass jar and placed ventral surface uppermost upon the operating-board. Anesthesia was maintained throughout the operation by means of a small ether cone. The hair was removed from the abdominal field by means of a sodium sulphide depilatory. The skin was then washed with chlorazene solution.

The area of operation was covered with a small laparotomy sheet. An incision of from 2 to 3 cm. in length was then made in the abdominal midline—the wound area being enlarged by the insertion of an eye speculum to secure retraction.

It has been found by Nicholas (1925) that the lumbodorsal method of approach is preferable to the linea abla incision, where fetuses are to be operated *in utero*. He states that by means of the lumbodorsal incision excessive visceral manipulation is avoided, a much stronger repair is possible, and the mammary glands of the mother are uninjured, thus allowing a higher probability of the rearing of the litter. From a strictly surgical standpoint, the superiority of the operation as devised by Nicholas is evident. However, when all fetuses are to be injected, a single ventral incision is preferable.

One horn of the uterus with its inclosed fetuses was carefully lifted out of the peritoneal cavity and the fetuses injected intraperitoneally. The injection needle, of 27 gauge (attached to a 1 cc. tuberculin fournier), was inserted through the uterine wall and the abdominal wall of the fetus. In performing fetal injections, from 0.02 to 0.05 cc. of freshly macerated pituitary substance was administered. The procedure was then repeated, using the other uterine horn. Both horns were then replaced within the body cavity of the mother, and the wound in the latter's abdominal wall was repaired.

Strong repair is essential on account of the great distension of the pregnant animal and the consequent strain upon the abdominal musculature and aponeuroses. One row of continuous catgut sutures (No. 000) secured the body wall, and a second row, discontinuous, closed the skin incision. The skin was then washed with chlorazene solution and the animal returned to its cage.

Prenatal injections were performed upon alternate days. The youngest animals injected were in the fifteenth day of gestation age. Sixteen, seventeen, and eighteen-day embryos were similarly injected. Twenty-one days was taken as the gestation period in all cases. The number of days elapsing from injection until birth, when subtracted from this sum, was considered to be the age of the fetuses at the time of injection.

Gonads of both sexes were preserved for sectioning at various ages of both injected and non-injected control animals. The tissues were fixed in Bouin's solution. Identical treatment of all tissues was rigidly practiced, since uniformity of result was essential.

The pituitary gland was prepared for injection in the following manner: The entire pituitary gland was excised from a freshly killed adult rat. It was then macerated under aseptic conditions in a glass mortar in 1 cc. of either normal saline solution or blood-plasma. When plasma was used, it was obtained from the donor of the gland.

TABLE I

SALINE PITUITARY SUSPENSIONS*

Age (Days)	No. of Litters	No. of Individuals
15	5	26
16	2	13
17	2	16
18	3	19
Prenatal		
Newborn	2	26

* Four litters supplied the control animals.

It was not deemed necessary to isolate the anterior lobe of the pituitary in these experiments. The desired result was a recognizable response to the pituitary hormones and nothing more. This is shown by the change in the gonad of the treated animal when compared with the untreated control.

Previous failure by other workers to obtain positive results with injections of the gland, single transplants, and pituitary feeding increases the importance of the method employed. As previously noted, both Klinger (1919) and Evans and Long (1922) reported negative results with saline-pituitary injections.

EXPERIMENTAL

Normal salt solution was used as the medium of injection of the animals included in Table I, while the plasma served a like function in the injection of the rats of Table III.

Blood-plasma was used as an injection medium in the second series of animals, in the hope of producing an earlier response of the gonad to the hormone. This was thought to be possible by introduc-

ing the macerated glandular material in a normal body fluid rather than in sodium chloride solution.

SERIES I

Prenatal injection.—Prenatal injection in no way hastened the differentiation of the gonad. It is thus evident that the gonad of the rat fetus is incapable of response to the pituitary hormone. In Table II an apparent advance of the injected fetal testis over the uninjected one is shown. However, the difference in the number of cells present is seen to be slight, amounting to 8.03 cells.

TABLE II

CELLULAR COUNTS IN THE GERMINAL EPITHELIUM

Age (Days)	Controls	σ*	$E\sigma\pm$	Injected	σ	$E\sigma\pm$
19 (prenatal)	22.6	2.600	0.2062	30.6	3.40	0.3014
At birth	23.6	1.713	.1257	30.0	8.32	.5612
1	30.5	4.127	.2887	30.0	2.541	.1916
5	34.7	3.721	.2806	33.4	4.78	.3604
6	33.6	5.320	.4010	33.2	3.242	.2445
9	36.3	1.320	.0995	37.3	.98	.0739
10	37.0	2.413	.1616	49.5	1.62	.122
12	48.1	1.788	.1348	59.5	7.31	.5513
13				57.4	3.61	.2722
14				64.4	3.422	.2580
15				109.4	8.02	0.6048
20	73.7	3.376	0.2546			

* σ = Standard deviation. $E\sigma$ = Probable error of standard deviation.

Since at 9 days of postnatal life the cells of the germinal epithelium of both experimental and control animals is shown to be practically identical, little importance can be attached to the apparent difference seen in the 19-day fetus.

Following the establishment of this fact, no fetal injections were made in the second series, where blood-plasma was used as the injection medium. Histologically, no difference between the testes of experimental and control animals is evident. In the female, the ovaries of injected and uninjected animals present an identical appearance. The follicles are plainly immature, and nothing comparable to the mature follicle is present. All traces of follicular fluid are, of course, absent.

Postnatal injection.—No evidence of precocious sexual differentiation was observed in either sex until the tenth day of postnatal life,

when the effect was most pronounced in the male. This effect evinced itself in an apparent increase in tubule length, as well as by a considerable thickening of the tubular wall. There is an evident increase in the size of the interstitial cells, and the tissue presents a dense and closely packed appearance. We attribute this closely packed appearance to the increase in length and diameter of the tubules.

It was felt that a check upon the histological picture presented at various ages was needed. To effect such a check, the cells contained within the germinal epithelium of the male animals were counted. Preparations from injected and control animals were placed under the high power of the microscope, and the cells counted

TABLE III

PLASMA PITUITARY SUSPENSIONS

Age	No. of Litters	No. of Individuals
Newborn	7	58
Newborn	2	16 (control)

by means of a counting ocular and tally. Table II shows the result of these counts, together with their standard deviations and probable errors.

The cells contained in cross-sections of forty tubules were counted for each of the ages represented in the table. Twenty counts were made for each age, where normal saline solution had been used as the medium of injection, and the counts then averaged. An equal number of counts were made for animals in which blood-plasma served as the injection medium. This average was then taken. The two averages were then added and the sum divided by two. The resulting average was recorded in the table as the number of cells present in the germinal epithelium of animals of that specific age. Gaps in the table indicate insufficient material to follow out this procedure.

If the cellular counts be considered, there is but a slight advance in maturity of the injected over the uninjected animals at 10 days of age. A succeeding increase, however, in the number of cells present is seen from the tenth day onward. At 20 days the difference is

very great, 33 per cent. Here the control animals have an average of 73.7 cells present in the germinal epithelium; while the injected animals show an average of 109.4 cells present at 15 days of age. Insufficient experimental material was available to make the table complete.

Figures 3 and 5 show testes from 20- and 10-day normal uninjected control animals. The scattered interstitial cells and small tubules are characteristic. Figure 8, from a 5-day injected animal,

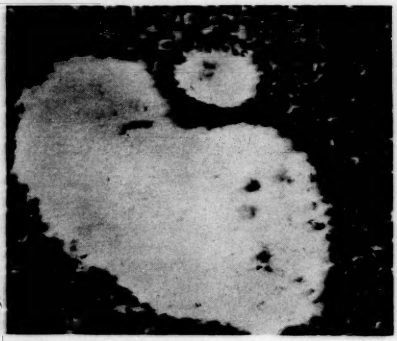

FIG. 1

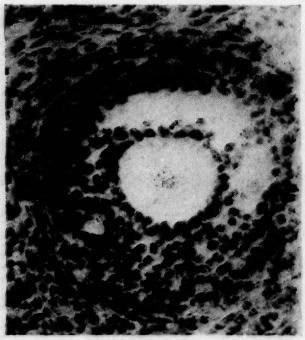

FIG. 2

FIG. 1.—Section through an ovarian follicle of a 15-day injected animal. Note the extent of the follicular wall together with the close packing of cells of the follicular epithelium. ×300.

FIG. 2.—Section through an ovarian follicle of a 20-day uninjected control animal for comparison with Figure 1. ×300.

shows a similar histological structure. At 12 days the structure of the testis is altered, and the closely packed arrangement of the tubules is evident. Figure 4, from a 15-day-old injected animal, shows this arrangement quite clearly, and, when compared with the histological picture presented by Figure 1, indicates the striking differentiation which has taken place.

In the ovary, the earliest advance in maturity of the injected animals over the uninjected controls appeared at approximately the fifteenth day. No corpora lutea were present at this stage, but the follicles are larger in diameter and are more nearly approaching maturity than in the normal animal, if judged by the amount of

follicular fluid present. In no case were corpora lutea observed, nor could eggs be seen within the lumen of the sectioned tube.

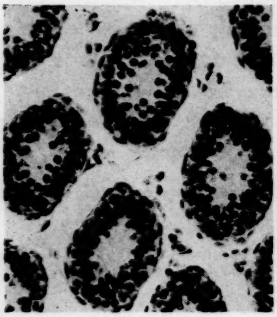

FIG. 3

FIG. 4

FIG. 5

FIG. 6

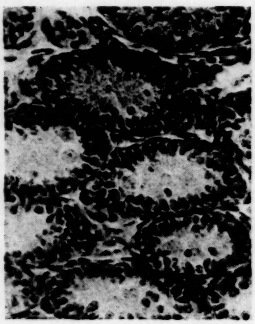

FIG. 7

FIG. 8

FIG. 3.—Section through the testis of a normal 20-day-old rat.
FIG. 4.—Section through the testis of a 15-day-old injected animal.
FIG. 5.—Section through the testis of a 10-day-old control animal.
FIG. 6.—Section through the testis of a 9-day-old injected animal.
FIG. 7.—Section through the testis of a 12-day-old injected animal.
FIG. 8.—Section through the testis of a 5-day-old injected animal.
All figures at the same magnification, ×300.

A comparison of Figures 1 and 2 shows the effect of pituitary injection in the female. In the 15-day injected animal the follicles are much larger than in the 20-day-old control. The increase in the amount of follicular fluid present indicates a much more mature follicle than that presented by the older control animal.

On the basis of the foregoing results, the physiological effect of the pituitary hormone upon the gonad first shows itself in both sexes between the tenth and the fifteenth days of postnatal life.

SERIES II

In the second series of animals, fresh blood-plasma was used as the injection medium. This was done, as previously noted, in the hope of producing an earlier response to pituitary injection in the gonad, by means of introducing the macerated glandular material in a normal body fluid rather than in the sodium chloride solution. In the light of the previous results, all injections were made postnatally. Injections were made daily, approximately 0.25 cc. of the suspension being administered at each treatment. In every case the blood-plasma to be used was obtained from the donor of the pituitary gland. The whole gland was macerated, as before, in 1 cc. of the fresh plasma, in order that the results obtained should be in every way comparable to those secured by employing the physiological saline solution. Fifty-eight animals, comprising seven litters, were used in this series of experiments. Two litters provided the controls. Fixation and sectioning of the gonads was done in a manner identical with that employed in the earlier series.

The results obtained were very similar to those previously described. The contrast between materials secured from 12-day-old rats indicates the amount of differentiation which has taken place. There is an increase in the number of cells composing the lining of the tubules, together with a thickening of the tubule wall and a tremendous lengthening of the tubule. Masses of cells occupy the lumen instead of a few scattered cells which are present in the uninjected animal. The interstitial cells are increased both in number and amount and fill completely the interstices between the tubules. Differentiation was first noted to occur at the ninth day in the injected animals, the picture presented by these rats being very similar to that obtained in the 20- to 30-day controls. The first evidence of differentiation is a closer packing of the cells than is present in the normal uninjected animal. Figure 6, when compared with Figures 5 and 3, shows the amount of differentiation which is secured in the 14-day-old injected forms.

The ovaries of this series did not show so great an advancement of maturity as was noted in Series I. However, if the amount of follicular fluid present be significant, the injected animals of 15 days of age show a more advanced follicular maturity than controls of the same age.

Evans and Simpson (1928) have shown that when the pituitary gland is extracted in an acid medium, the maturity-provoking hormone is dominant; while in alkaline medium, the growth-producing hormone appears in the ascendency. This fact may account for the apparent discrepancy in results in regard to the ovary, since the blood-plasma of the rat is undoubtedly more alkaline than normal saline solution. If, however, this were the case, maturity should be retarded also in the male. This does not occur.

Other than in respect to the ovary, the results obtained by the use of fresh blood-plasma as an injection medium are identical with those obtained by using the normal salt solution. The earliest response to pituitary injection evinced itself at the tenth day of postnatal life, in the male, and in the female between the tenth and fifteenth days. The results obtained by means of employing fresh blood-plasma as a medium of pituitary injection thus corroborate those secured by using normal saline solution for a like purpose. Certainly, no difference is discernible, if the age of the animal at the time of reaction to injection be taken as the criterion.

DISCUSSION

Prenatal injection of pituitary substance in no way affects the gonad of the fetal rat. An apparent increase in the number of cells contained within the germinal epithelium of the injected animals takes place. However, this difference in cellular counts between the injected and the control animals may be ignored, since at 9 days of age the counts are practically identical. Study of histological sections reveals no difference between the gonads of animals subjected to pituitary injection and the controls.

Postnatal injection of the pituitary gland brings about a marked differentiation in the gonad, when compared with that of uninjected control animals. This differentiation evinces itself between the tenth and fifteenth days of postnatal life in both sexes. In the male the advanced maturity of the gonad shows itself in a considerable

increase in the number of cells in the germinal epithelium, together with an entirely different histological picture from that observable in the controls. The tubular diameter is increased, and the tubules are crowded together, resulting in a complete obliteration of the intertubular spaces present in the normal gonad. This altered appearance may be attributed to growth in length and diameter of the tubules, together with an increase in the size of the interstitial cells. In the female, the advanced maturity is shown by an increase in follicular size as well as by the amount of follicular fluid present within the individual follicles. The follicles appear entirely normal, but strikingly more mature than the follicles of control animals of the same age.

It was not the purpose of the present study to effect complete maturation, as has been done by Smith and Engle (1927), but rather to utilize the maturation effect upon the gonad as an indicator of the early effect of the pituitary secretions. Hence, the continuation of the experiments beyond the point where indications of advanced maturity in the gonad were patent was unnecessary in this study.

It is evident from the work of Smith that the primary effect of pituitary grafts is upon the gonad, and that this effect, i.e., precocious maturation, affects the entire genital tract by reason of the increased activity of the sex glands. It was therefore deemed unnecessary in the present study, where the earliest response to the pituitary hormones was sought, to carry the observations beyond the responses evoked within the gonad.

The determination of the time at which the tissues of an organism will respond to endocrine injections has a most important bearing upon other branches of endocrinology. The failure of an organism to respond to certain specific hormones may, in some cases, be due entirely to the age of the organism employed. In an older animal, responses might be evoked which fail to show themselves in younger stages of development.

Conclusions as to the time of early activity of various of the endocrine glands, based upon cytological evidence, may be questioned from the standpoint of their physiological correlation. If the tissues of a developing organism are incapable of response, the presence of the products of internal secretion is relatively unimportant.

Since the gonad of the rat fetus is incapable of response to the secretions of the pars anterior of the pituitary gland, the problem of placental transmission of this hormone is additionally complicated. Even if the placental transmission of the hormone of the pars anterior is a fact, no indication of such transmission can be noted in the responses of the gonad of the fetus.

The failure of the tissues of the fetus to respond to the pituitary hormone may have an important bearing upon the extent to which the maternal endocrine secretions can be said to influence the developing embryo. Theories relating to prenatal endocrine influence should be accepted with circumspection, until proof is brought forward to the effect that the fetus is capable of response to these specific hormones. Responses to certain hormonal secretions possibly may be evoked in the developing fetus; but until proof of such responses is produced, nothing of a definite character should be stated regarding them.

It is possible that the importance of the placental barrier in maintaining the independence of the embryo from maternal endocrine influence has been greatly overemphasized. The injection of glandular extracts into the mother, with negative results as regards discernible effect upon the fetus, cannot be considered conclusive proof of the impermeability of the placenta to products of endocrine metabolism.

The absence of tissue reaction to endocrine secretions may be considered specific indication of the independence of embryonic development. Certainly, in the case of the pituitary, either excessive maternal secretion or deficient secretion, cannot greatly influence intrinsic development of the fetus.

The conditions produced in these experiments cannot be considered similar to those obtaining in the freemartin, as described by F. R. Lillie (1917). In the freemartin the fusion of the vessels of the chorion places the fetal blood-streams in direct communication. Thus the zygotically determined female is subjected to the sex hormones elaborated by the male fetal twin. In these experiments the conditions are quite dissimilar. The fetus is subjected to periodic injections of pituitary substance from a mature animal and fails to react to it.

CONCLUSIONS

1. The gonad of the albino rat shows accelerated differentiation following injection of the macerated whole pituitary gland.

2. This reaction may be obtained by the use of either normal saline or fresh blood-plasma of the rat as a suspending medium for the macerated glandular material.

3. The gonad of the fetal albino rat is incapable of response to pituitary injection.

4. Hastened differentiation of the gonad first shows itself, *in both sexes*, between the tenth and the fifteenth days of postnatal life.

5. Hastened differentiation of the gonad produced by pituitary injections is more pronounced in the male.

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THE SOMATIC EFFECTS OF TEMPERATURE ON A HOMOZYGOUS RACE OF DROSOPHILA[1]

(Six figures)

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INTRODUCTION

THE present paper is a quantitative study of the effects of temperature on various somatic structures of a homozygous race of *Drosophila*. Determinations were made of the size of various parts of the body, of hair number, and of the length of the immature period. The technique of culturing *Drosophila* so that uniform results may be obtained in repeating the experiments is given special attention.

MATERIALS AND METHODS

The flies used in all of the experiments were of a homozygous red, forked, bar stock obtained from Dr. Zeleny and listed by him as "low selected bar 80 forked, number 1165.1." This stock has been inbred for many generations.

Banana agar was used as the food medium for the flies. Ripe and fairly large bananas were mashed through a potato-ricer and then mixed with an equal part of water. The mixture was stiffened with 1 per cent agar and was heated at a temperature of 90° C. for about 10 minutes. Eight-dram vials were used in all experiments. A small strip of absorbent paper toweling was placed vertically in each vial. The vials were stoppered lightly with cotton plugs and were sterilized for 15 minutes in an autoclave. Approximately 9 grams of the food medium was placed in each vial; and after it had cooled somewhat, but before it had solidified, a pinch of Yeast Foam was added. The yeast was permitted to grow for at least 24 hours at room temperature before the parents were put into the vials. After the yeast has grown for this period of time, the vials have a

[1] Contribution from the Zoölogical Laboratory of the University of Illinois, No. 378.

fresh yeasty odor, and the food in this condition is very conducive to oviposition.

The parents were raised at 25° C. in 40-dram bottles. The flies in the stock bottles were not raised under overcrowded conditions, and as a result the offspring which emerged were large in size. Large, well-fed flies consistently lay more eggs than small flies. Pair matings were made in all cases. After the matings were made, the flies were placed on fresh food for at least 24 hours, to give them ample time to recover fully from the effects of etherization. After this the flies were shaken into the experimental vials and were changed to fresh food every 3 hours. These experiments were all started at 7:00 A.M., and four sets of vials were used. After each 3-hour egg-laying period at 25° C., the vials with the eggs were immediately removed to the temperature at which they were kept for the experiments. The parents were changed from one vial to another in such a way that eggs from the same parents were placed at each temperature; thus the differences noted in the offspring hatching at the different temperatures could not be attributed to differences in the genetic constitution of the parents. With this procedure the same number of eggs was placed at each temperature, and thus the factor of differences in the density of population at different temperatures was practically eliminated. The offspring at each temperature were removed at 6-hour intervals (at 7:00 A.M., 1:00 P.M., 7:00 P.M. 1:00 A.M.), and records were kept of the numbers of males and females hatching during each removal period. The flies were kept on fresh food until all had hatched, and then were weighed.

The weight determinations were made with a balance, accurate to milligrams. The flies were weighed while etherized, and separate weight determinations were made for males and females. For the counting of facets and hairs and the measurement of size, the flies were placed in depressions on the wax in a Syracuse dish. The wings were removed with a small scalpel before the measurements were made.

Four temperatures, namely, 29.5°, 27°, 22.5°, and 17° C., were secured with incubators. The 29.5° C. and the 27° C. incubators were kept in a warm-room which was maintained at a temperature of 25° C. The 22.5° C. and the 17° C. incubators were in a cold-

room which was kept at a temperature of about 15.5° C. This cold-room, which showed a temperature variation of 1.0° C., served as one of the temperatures for the experiments performed. The air in the 27° C. and the 17° C. incubators was kept in circulation by means of electric fans, and thermograph records of these show that the temperature fluctuated but very little. In the 29.5° C. and the 22.5° C. incubators, which were not equipped with electric fans, the temperature showed a variation of approximately 0.7° C.

Since the temperature intervals were large, these minor fluctuations did not appreciably affect the final results. It was not possible to make the determinations of thorax length and head width as accurately as the determinations of wing length and wing width. The ocellar hair counts were easily and accurately made, but there was always some error involved in making the circumocular hair counts. The unavoidable error in the weight determinations, due to the amount of food eaten by the flies before weighing, was checked by the wing length and thorax length determinations which were made afterward.

EXPERIMENTAL DATA

TEMPERATURE EFFECT ON WEIGHT OF FLIES

Preliminary experiment.—In the spring of 1923 a preliminary test was carried out to determine the effect of temperature on the weight of *Drosophila*. The flies used in this test were raised at 16° C. and 27° C. by Dr. Zeleny in an experiment to determine the effect of temperature on the rate of mutation. The stock was the same race of *Drosophila* used in all the experiments recorded in this paper. No records of the number of flies per vial were made.

The weights are given in Table I. The flies raised at 16° C. show a decided increase in weight over those raised at 27° C., and the differences in weight are of sufficient magnitude to be significant. The females at both temperatures are heavier than the males. It was thought that possibly the smaller size of the flies raised at 27° C. might be due to overcrowding, and therefore in the experiments performed afterward care was taken to record the exact number of offspring per vial.

Temperature effect on weight of flies with ten pairs of parents per vial.—Three sets of experiments were performed at four different

temperatures, and the averages of the three experiments are shown in Table II. At 29.5° C. the flies are heavier than those raised at 27° C., and this can be accounted for by the fact that the flies at 27° C. were raised under more overcrowded conditions than those raised at 29.5° C. However, the data of these experiments are consistent in showing that between 17° C. and 27° C. there is a decrease in weight with an increase in temperature.

Temperature effect on flies with two pairs of parents per vial.—These experiments were repeated, and four experiments were performed with only two pairs of parents per vial, the results of which are given in Table II. In these experiments the flies raised at 27° C. were heavier than those raised at 29.5° C., despite the fact that more flies hatched out at the former than at the latter temperature. Below 17° C. there is very little increase in weight. Figure 1 shows the relationship between weight and temperature of the flies with two pairs of parents per vial, and the curves for males and females both indicate that between 29.5° C. and 17° C. there is an increase in weight with a decrease in temperature.

TABLE I

TEMPERATURE EFFECT ON WEIGHT OF FLIES

	Temperature at 16° C.			Temperature at 27° C.		
	No. of Flies	Average weight per Fly (Milligrams)		No. of Flies	Average weight per Fly (Milligrams)	
		Females	Males		Females	Males
	362	1.34	0.98	676	1.02	0.66
	212	1.45	1.00	268	0.90	.61
	160	1.30	0.96	435	.74	.56
	144	1.26	0.97	287	.84	.63
	220	1.37	1.04	324	.80	.60
	202	1.34	0.93	.....	.....	.....
Total and weight average.....	1,300	1.35	0.98	1,990	0.89	0.61

Temperature effect on flies with three pairs of parents per vial.—These experiments were again repeated during the summer of 1927, and the results are given in Table II. A large number of flies were raised at three temperatures, and the results indicate again that there is an increase in weight with a decrease in temperature.

TEMPERATURE EFFECT ON SIZE

Measurements of the length of the thorax, of wing length, of wing width, and of head width were made in order to determine their relationships to each other and to the number of hairs and to facet count. The flies examined were taken at random from experiments 4 (*a*, *b*, *c*), and at each temperature flies were taken from two or from all three of these experiments.

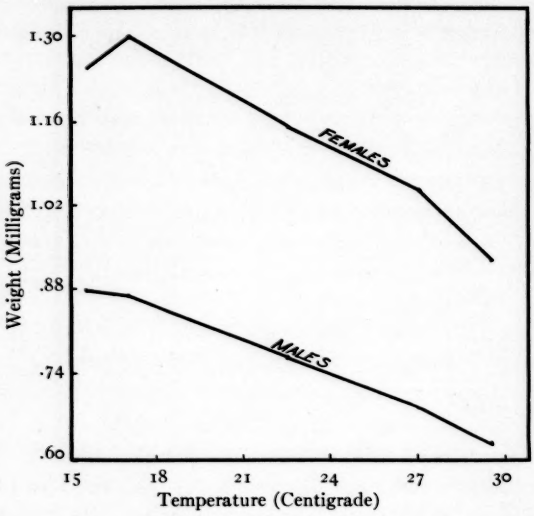

FIG. 1

Thorax length.—The thorax length was determined by measuring the distance from the anterior end of the dorsal side of the thorax to the posterior tip of the scutellum. The results are given in Table III. Figure 2, showing the relationship between thorax length and temperature for both males and females, indicates that between 15.5° C. to 17° C. there is very little change in thorax length but that between 17° C. and 29.5° C. there is a steady decrease in thorax length with increase in temperature.

Wing length and wing width.—The wing length given is the distance between the anterior cross-vein and the tip of the III-longitudinal vein. The wing-width measurements were made across the

wing, from the tip of the V-longitudinal vein to the opposite side in a straight line at right angles to the long axis of the wing. Both sets of measurements show that a decrease in temperature is correlated with an increase in wing width and wing length between

TABLE II

EFFECT OF TEMPERATURE ON WEIGHT OF FLIES WITH 2, 3, AND 10 PAIRS OF PARENTS PER VIAL

Experiment No.	No. of Parents	Temperature (Degrees Centigrade)	No. of Flies	Average No. per Vial	Average Weight per Fly (Milligrams)	
					Females	Males
2 (*a*, *b*, *c*)	10 pairs	29.5	1,110	13.8	0.77	0.57
		27.0	3,627	37.8	.73	.54
		22.5	3,683	38.7	.79	.58
		17.0	3,108	33.7	.93	.70
4 (*a*, *b*, *c*, *d*)	2 pairs	29.5	626	8.4	0.93	.63
		27.0	1,721	12.6	1.05	.69
		22.5	1,713	13.6	1.15	.77
		17.0	1,650	12.4	1.30	.87
		15.5	1,490	12.0	1.25	.88
5	3 pairs	27.0	7,470	20.7	1.05	.68
		22.5	7,818	21.7	1.15	.76
		17.0	7,438	20.7	1.21	0.90

TABLE III

SIZE MEASUREMENTS IN MILLIMETERS

	29.5° C.	27.0° C.	22.5° C.	17.0° C.	15.5° C.
Average thorax length (females)	0.895 ± .003	0.956 ± .002	1.021 ±0.002	1.067 ±0.002	1.066 ±0.002
Average thorax length (males)	.790 ±0.002	.836 ±0.002	.903 ±0.002	.958 ±0.002	.962 ±0.002
Average wing length (females)	1.319 ±0.004	1.378 ±0.002	1.478 ±0.002	1.654 ±0.002	1.680 ±0.002
Average wing length (males)	1.141 ±0.003	1.202 ±0.001	1.300 ±0.002	1.493 ±0.002	1.521 ±0.002
Average wing width (females)	.855	.921	.958	1.040	1.045
Average wing width (males)	.766	.816	.869	0.969	0.984
Average head width (females)	.679	.697	.736	.788	.790
Average head width (males)	0.634	0.655	0.694	0.744	0.755
*No. of females examined	70	116	97	105	70
*No. of males examined	70	106	97	105	70

* This same number of flies was examined for all the determinations given in the following tables.

17° C. and 15.5° C., while the thorax lengths do not increase between these temperatures. Figure 3 shows the wing-length–temperature relationship, and the curves for both the males and females appear to be straight-line curves.

Head width.—The head-width measurements, which were made from the dorsal side, represent the diameter of the whole head. These measurements are also given in Table III, and the results show that there is a decrease in head size with an increase in temperature.

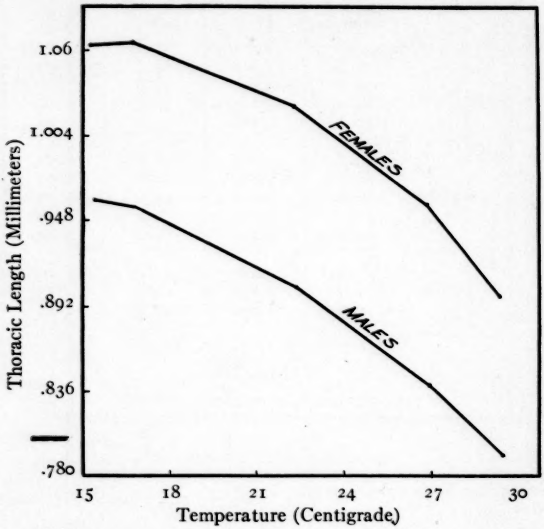

FIG. 2

RELATIONSHIPS OF SIZE MEASUREMENTS

Differences between males and females.—The weight-sex coefficients (Table IV) are not consistently affected by temperature. However, the coefficients of the smaller flies, which had ten pairs of parents per vial, are larger than those of the large flies at all temperatures. From this we may conclude that the larger flies raised under optimum conditions show greater sexual dimorphism than the smaller flies. Data to be published in another paper, of small flies of this same stock, where the flies were raised under very over-

crowded conditions, substantiate this conclusion, for the weight-sex coefficients of some of these small flies are as high as 0.88. The

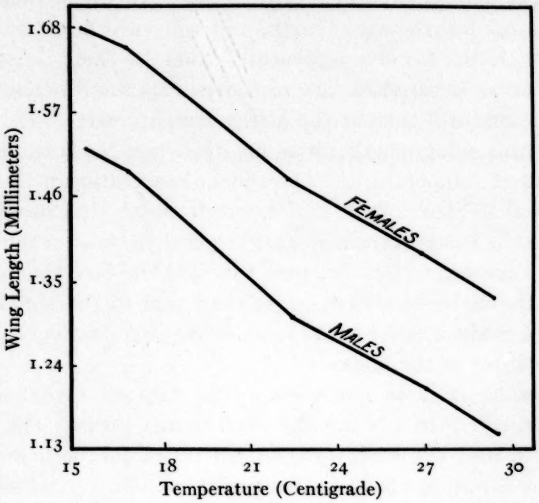

FIG. 3

TABLE IV

SIZE RELATIONSHIPS

	29.5° C.	27.0° C.	22.5° C.	17.0° C.	15.5° C.
Thorax-sex coefficient	0.88	0.87	0.88	0.90	0.90
Wing-length–sex coefficient	.86	.87	.88	.90	.90
Wing-width–sex coefficient	.90	.88	.91	.93	.94
Head-width–sex coefficient	.93	.94	.94	.94	.95
Thorax–wing-length ratio (females)	.68	.69	.69	.64	.63
Thorax–wing-length ratio (males)	0.69	0.69	0.69	0.64	0.63
Thorax–wing-width ratio (females)	1.05	1.04	1.06	1.03	1.02
Thorax–wing-width ratio (males)	1.03	1.02	1.04	0.99	0.98
Thorax-head ratio (females)	1.32	1.37	1.39	1.35	1.35
Thorax-head ratio (males)	1.24	1.28	1.30	1.29	1.27
Head–wing-length ratio (females)	0.51	0.51	0.50	0.48	0.47
Head–wing-length ratio (males)	.55	.54	.53	.50	.49
*Wing-length–thorax "r" (females)	.84	.77	.66	.59	.73
*Wing-length–thorax "r" (males)	.69	.66	.62	.60	.49
Weight-sex coefficient (10 pairs of parents)	.74	.74	.73	.75	.....
Weight-sex coefficient (2 pairs of parents)	0.68	0.66	0.67	0.67	0.70

* "r" = correlation coefficient.

weight-sex coefficients are much lower than those of any of the size measurements, and this is probably due to the fact that the abdomens of the females are much larger than those of the males.

The thorax length, wing-length, and wing-width sex coefficients are higher at the lower temperatures; that is, there is less sexual dimorphism as far as these size measurements are concerned at the lower temperatures than at the higher temperatures.

The thorax–wing-length ratios are the same for both males and females at all temperatures. The thorax-head ratios of the females are larger than those of the males, which means that in relation to the thorax the heads of the males are larger than those of the females. There is some connection between this and the fact that the facet count of the males is always larger than that of the females. The thorax–wing-width ratios of the females are larger at every temperature than those of the males.

Temperature effect on size ratios.—The data show that temperature is ineffective in altering the head-thorax ratios. The thorax–wing-length and the head–wing-length ratios for both males and females are lowest at the lower temperatures. Two conclusions suggest themselves: either the rate of development of the wing is faster at the lower temperatures than that of the thorax or the effective period of wing formation at the lower temperatures may be lengthened more than the effective period for the formation of the thorax.

Wing-length–thorax-length correlation coefficients.—With the exception of the flies raised at 17° C., the females show a higher correlation coefficient than do the males. With one exception, the correlation coefficients of the flies raised at the higher temperatures are higher than those of the flies raised at the lower temperatures.

TEMPERATURE EFFECT ON HAIR NUMBER

Hair-number counts were made of the flies raised at the five temperatures. The circumocular hairs are those that are grouped in almost a circle around the faceted area of the eye, and this is a clearly marked-off area. The ocellar hairs are arranged in two clearly defined rows, in the area marked off by the two lateral and median ocelli.

Ocellar hair number.—Table V shows that there is an increase in

ocellar hair number with a decrease in temperature for both males and females. There are no consistent sexual differences, and in this respect ocellar hair number is different from most other characters studied.

Figure 4 shows the ocellar-hair–temperature relations. The curves are quite different from the curves for the other characters mentioned above, but they have somewhat the same appearance as

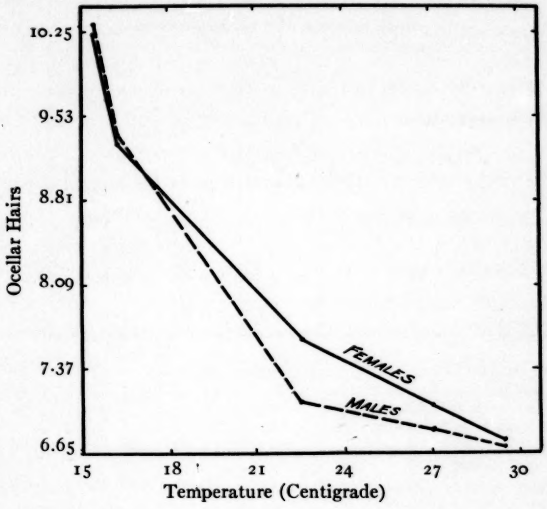

FIG. 4

the egg-larval-pupal developmental curves shown in Figure 6. The curves show that the lower temperatures produce more ocellar hairs per degree centigrade than the higher temperatures. Because of the limited number of temperature intervals for these experiments, the exact temperature–ocellar-hair relations cannot be worked out. However, the data show that there is a marked increase in hair number per degree centigrade at the lower temperatures, and this increase is much greater than the increase in the size of the flies, as is demonstrated by the thorax– and wing-length–ocellar hair ratios shown in Table V.

A number of other investigators, working on the effect of selection

on the scutellar bristles, also experimented with the effect of temperature on bristle number. MacDowell (1915) raised flies at different temperatures and concluded that temperature was not effective in altering bristle number. Reeves (1916), in connection with selection work, concluded that temperature did not affect bristle number. Payne (1920) obtained slight temperature effects on bristle

TABLE V

HAIR NUMBER AND RELATIONSHIPS

	29.5° C.	27.0° C.	22.5° C.	17.0° C.	15.5° C.
Circumocular hairs (females)	52.7 ± 0.22	53.90 ± 0.25	57.60 ± 0.18	62.30 ± 0.22	60.70 ± 0.11
Circumocular hairs (males)	51.70 ± 0.21	53.00 ± 0.25	57.40 ± 0.17	61.20 ± 0.20	61.00 ± 0.21
Circumocular-hairs–sex coefficient	0.98	0.98	0.99	0.98	1.00
Ocellar hairs (females)	6.74 ± 0.06	7.03 ± 0.05	7.61 ± 0.06	9.30 ± 0.08	10.23 ± 0.12
Ocellar hairs (males)	6.66 ± 0.06	6.82 ± 0.05	7.06 ± 0.05	9.36 ± 0.08	10.30 ± 0.10
Ocellar-hairs–sex coefficient	0.99	0.97	0.93	1.00	1.00
Thorax–ocellar-hairs ratio (females)	7.18	7.35	7.82	6.16	5.63
Thorax–ocellar-hairs ratio (males)	6.41	6.63	6.91	5.53	5.05
Wing-length–ocellar-hairs ratio (females)	10.60	10.50	10.50	9.60	8.90
Wing-length–ocellar-hairs ratio (males)	9.26	9.53	10.20	8.60	8.00
Thorax–circumocular-hairs ratio (females)	0.92	0.96	0.96	0.93	0.95
Thorax–circumocular-hairs ratio (males)	0.83	0.85	0.85	0.85	0.85

number, finding that the mean bristle number of flies reared at 10°–13° C. was 2.34, and that of flies reared at 23°–26° C. was 2.14. His differences, although rather small, are in the same direction as the results under discussion in this paper.

Circumocular hairs.—Figure 5, showing the circumocular-hair–temperature relation, indicates that these hairs also increase in number with a decrease in temperature, but these curves are straight-line curves and are quite different from the ocellar-hair–temperature curves. The curves show that there are no consistent sexual differ-

ences. The circumocular hair counts and relationships are shown in Table V. The differences for each temperature interval are not very large but are large enough to be statistically significant. The

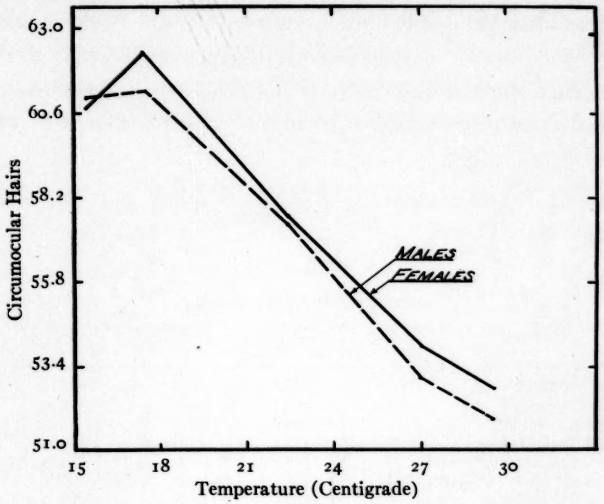

FIG. 5

thorax-circumocular ratios are much more constant than the thorax–ocellar-hair ratios, thus indicating that the former show a direct relationship to the thorax length, and increase or decrease in number with the increase or decrease in the size of the flies.

TEMPERATURE EFFECT ON TEETH OF SEX-COMBS

The sex-comb is confined to the male, and is situated on the first tarsal segment of the first pair of legs. The teeth comprising the sex-comb are really modified bristles. Table VI shows no consistent

TABLE VI

TEMPERATURE EFFECT ON NUMBER OF TEETH OF SEX-COMBS

29.5° C.	27.0° C.	22.5° C.	17.0° C.	15.5° C.
9.34±0.07	9.53±0.05	9.60±0.05	9.46±0.05	9.36±0.06

alteration of the number of teeth of sex-combs with change of temperature.

Hoge (1915) carried out selection experiments on the teeth of sex-combs and was able to produce races of flies with high and low numbers of teeth.

COEFFICIENTS OF VARIABILITY

The coefficient of variability is the ratio expressed in percentage of the standard deviation to the mean. It is calculated by the formula:

$$\text{C. V.} = \frac{100 \text{ Standard deviation}}{\text{Mean}} .$$

TABLE VII

COEFFICIENTS OF VARIABILITY

	29.5° C.	27.0° C.	22.5° C.	17.0° C.	15.5° C.
Thorax (females)	5.0	3.2	2.3	3.0	2.6
Thorax (males)	3.7	3.0	3.5	2.9	2.6
Wing length (females)	3.7	2.4	2.3	1.9	1.9
Wing length (males)	3.8	1.9	2.3	2.0	1.7
Circumocular hairs (females)	5.3	7.5	4.7	5.5	4.5
Circumocular hairs (males)	5.1	7.2	4.3	5.0	4.2
Ocellar hairs (females)	11.7	8.5	11.1	13.3	14.4
Ocellar hairs (males)	12.1	11.5	13.5	14.3	11.8
Sex-combs	9.0	8.4	7.6	7.6	7.7

Table VII shows the coefficients of variability of the various measurements and counts made. Although temperature does not appear to affect the degree of variability very consistently, yet in every case, with the exception of ocellar hair number, the variability at 15.5° C. is less than at the higher temperatures. None of the coefficients of variability of body measurements show any consistent sexual differences.

TEMPERATURE EFFECT ON THE IMMATURE PERIOD (EGG-LARVAL-PUPAL)

Loeb and Northrop (1917) and Krafka (1919) worked out the length of the immature period of *Drosophila* at close temperature intervals but did not make separate determinations for males and females. Table VIII shows data from experiments made for this purpose. The flies were removed at 6-hour intervals, and the sexes

were separated. The average immature period is the average time taken by all the flies hatching in the vials, while the minimum immature period is the average of the immature period of the first males and first females to hatch in each vial.

The data in Table VIII show that overcrowding does increase the length of the immature periods, for the flies hatching from vials which had ten pairs of parents per vial had longer immature periods than those hatching from vials with two pairs of parents per vial.

TABLE VIII

TEMPERATURE EFFECT ON IMMATURE PERIOD (EGG-LARVAL-PUPAL), IN HOURS

Temperature (Degrees Centigrade)	Length of Immature Period				No. of Flies per Vial	No. of Pairs of Parents per Vial
	Females		Males			
	Minimum Period	Average Period	Minimum Period	Average Period		
29.5	176.5	193.5	185.0	202.8	13.8	10
27.0	190.3	228.3	195.0	234.4	37.8	10
22.5	228.5	276.5	234.7	279.8	38.7	10
17.0	501.0	575.0	518.6	595.3	33.7	10
29.5	172.2	174.6	178.8	181.1	8.4	2
27.0	177.9	181.2	182.8	185.9	12.6	2
22.5	210.2	218.6	216.5	223.3	13.6	2
17.0	493.9	508.2	511.0	522.3	12.4	2
15.5	644.2	665.1	664.8	687.7	12.0	2

This table also shows that the females have shorter immature periods at all temperatures, and that the differences in hatching-time between males and females increase with a decrease in temperature, between the experimental temperatures of 29.5° and 15.5° C. At 29.5° C. the difference in hatching-time between males and females is greater than at 27° C. Figure 6 shows the temperature-time relationship of the minimum immature period of the flies where two pairs of parents were used.

DISCUSSION

TEMPERATURE AS A FACTOR IN THE MECHANISM OF DEVELOPMENT

Many organisms show variations in their structural characteristics which may be considered a direct response to changes in temperature. It would be impossible to review the whole field of

literature on this subject, and so only those experiments which have a bearing on the problem of the paper will be briefly mentioned.

Merrifield (1891) and Reiff (1909) changed the color pattern of butterflies by means of temperature. Standfuss (1895) found a reduction in the size of butterflies as a result of rearing the larvae at high temperatures.

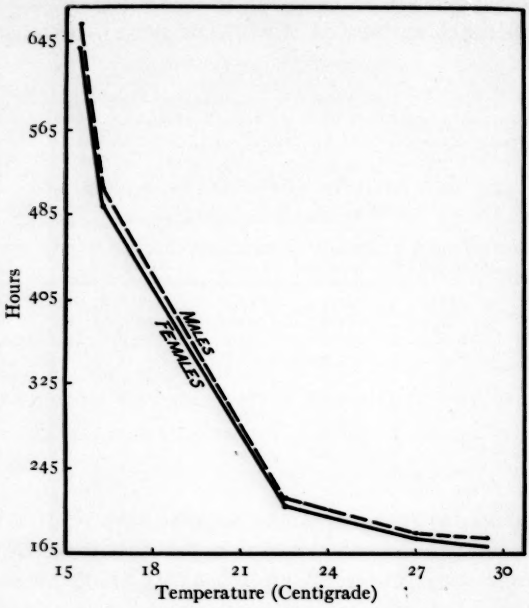

FIG. 6

A number of investigators have published results on the effects of temperature on fishes. Collett (1880) and Heincke (1880) found differences in structure and size measurements of fishes which may be attributed to the effects of temperature. Jordan (1921) found that the northern genera of the rock cod have more vertebrae than its nearest relatives found in tropical waters. Hubbs (1922 and 1924) found in a number of species of fishes that the number of vertebrae and other meristic characters varied inversely with the temperature of the water in which the fish were raised. Thus, most

of the data on the effects of temperature on fishes show that the number of vertebrae, the number of rays, and in some cases even the actual size varies inversely with the temperature of the water in which they develop, and these results agree with those obtained from experiments on *Drosophila*.

Allan (1874 and 1876) found that as a general rule the size of warm-blooded vertebrates decreases in going from the south to the north, and that in the case of birds this reduction amounts to 10 or 15 per cent of the maximum size of the species.

A few investigators have shown that certain characters increase in size or number at high temperatures. Sumner (1909, 1911, and 1915) reported that mice grown at high temperatures showed longer ears, feet, and tails than the controls; but he found no consistent differences in the body weights at high or low temperatures. Roberts (1918) determined the effects of temperature on the vestigial wing of *Drosophila* and found that high temperatures increased the size of the wing and that the increase was greater among the males than among the females. Cox (1923) has shown that a stickleback, *Apeltes quadracus*, shows an increase in the number of dorsal spines in water characterized by a reduced salinity and an increased temperature.

Considerable work has been done on the somatic effects of temperature on the eye of *Drosophila*. Luce (1926) found that the mean facet number of infrabar, an allelomorph of bar eye in *Drosophila*, varies directly with the temperature at which the flies are raised. Krafka (1919), Seyster (1919), Zeleny (1923), R. K. Hersh (1924), and A. H. Hersh (1924) have shown with various stocks of *Drosophila* that the number of facets of the eye varies inversely with temperature. Driver (1926) has shown that facet number is increased in the same proportion as is the length of the effective period of facet formation.

CONSIDERATION OF THE MEANS BY WHICH TEMPERATURE AFFECTS THE FLIES

As shown in this paper, the size of the flies and the hair number vary inversely with temperature. A number of explanations suggest themselves to account for this temperature effect, but no single explanation seems to satisfy all the conditions.

Since the immature period of *Drosophila* varies inversely with temperature, the flies at the lower temperatures have a longer period to feed and grow and thus attain a greater size. The obvious objection to such an explanation is that the prolonged larval period at low temperatures merely compensates for the retarded rate of metabolic processes, and thus we would not expect the end-product at low temperatures to be greater than at the high temperatures. However, it is quite evident that, although the growth rate is slowed down at the lower temperatures, it must be maintained for a longer period of time, for the flies are larger at the lower temperatures. Hubbs (1926) gave this as an explanation for the increased number of vertebrae, and other meristic characters of fishes raised at low temperatures; and he mentioned the experimental work of Lehenbauer (1914), who "found that the rapid initial rate of growth of plant seedlings under high temperatures is not long maintained, whereas the slow initial growth under low temperatures is maintained even under prolonged exposure."

Since increase in ocellar hair number is greater than the increase in the size of the flies, and since the length of the wings increases between 17° and 15.5° C. while the thorax length does not increase between these temperatures, and since the number of teeth of the sex-combs is not affected at all, temperature quite evidently has a differential effect upon the growth of the various structures of the fly.

ACKNOWLEDGMENTS

I wish to thank Professor Charles Zeleny for the opportunity of pleasant association in research and for his many helpful criticisms and valuable suggestions during the prosecution of the problem.

SUMMARY

1. The weight of the bar-eyed mutant of *Drosophila* varies inversely as the temperature at which the flies are raised. The males are consistently lighter than the females.

2. The weight-temperature, the thorax-length–temperature, and the head-width–temperature relations may be plotted as straight-line curves between 17° and 29.5° C.

3. The wing length and the wing width vary inversely with the

temperature at which the flies are raised, but their increase is greater at the lower temperatures than the increase in thorax length.

4. The thorax–wing-length ratios show no sexual dimorphism at any temperature.

5. The thorax–head-width ratios of females are larger than those for males at all temperatures.

6. There is less sexual dimorphism in the case of all size measurements at the low temperatures than at the high temperatures.

7. The smaller flies raised under overcrowded conditions show less sexual dimorphism in weight than do the flies raised under optimum conditions.

8. The wing-length–thorax-length correlation coefficients vary directly with temperature, and the coefficients of the females are higher than those of the males.

9. Circumocular hair number varies inversely with temperature. The circumocular-hair–temperature curve is a straight line.

10. Ocellar hair number also varies inversely with temperature, but the ocellar-hair–temperature curve is not a straight line.

11. There is no significant sexual difference in hair number.

12. Temperature does not consistently affect the number of teeth of sex-combs.

13. There are no consistent sexual differences in the variability of wing length and thorax length.

14. With the exception of ocellar hair number, the variability of all structures is least at the lower temperatures.

15. The immature period (egg-larval-pupal) of females is shorter than that of males at all temperatures, and the differences in hatching-time between males and females increase with a decrease in temperature.

16. Overcrowded conditions decrease the size of the flies and increase the length of the immature period.

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ACTION OF TWENTY-THREE OCTYL ALCOHOLS ON CARASSIUS AUREUS AND DIEMYCTYLUS VIRIDESCENS

(Four figures)

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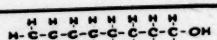
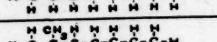
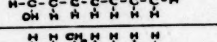
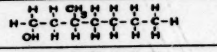
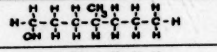
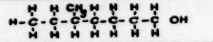
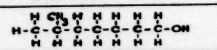
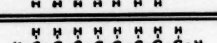
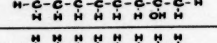
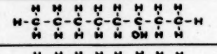
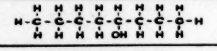
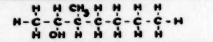
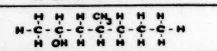
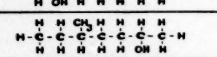
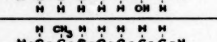
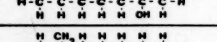
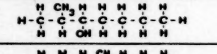
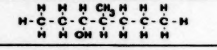
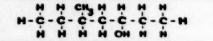
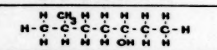
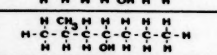
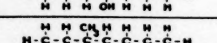
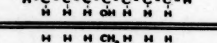
INTRODUCTORY

THE present investigation has been rendered possible through the courtesy and kind co-operation of Dr. E. Emmett Reid, professor of organic chemistry, Johns Hopkins University. Under Professor Reid's guidance, three candidates for the degree of Doctor of Philosophy—Messrs. G. L. Dorrough, H. B. Glass, and G. B. Malone—undertook for the subject of their dissertations the preparation and isolation in pure form of a series of isomeric octyl alcohols and the determination of their various chemical and physical constants. Theoretically, some fifty isomeric octyl alcohols are possible, and these chemists prepared twenty-three. Inasmuch as one of the most interesting subjects of pharmacology is the study of the relationship between chemical structure and physiological action of drugs and chemicals, the writer was very fortunate in obtaining the privilege of making a study of the various isomeric octyl alcohols thus secured, and grateful acknowledgment is hereby made to Professor Reid and his pupils for furnishing samples of these valuable compounds. The complete data on the subject, dealing with all kinds of zoöpharmacological and phytopharmacological experiments, will be published in a pharmacological journal. In the present paper it was deemed desirable to report several series of experiments which are of particular interest to zoölogists. These were performed on goldfish, *Carassius aureus*, and the common newt, *Diemyctylus viridescens*.

METHODS OF STUDY

A series of twenty-three isomeric primary, secondary, and tertiary pure octyl alcohols is included in the present study. Their names and structural formulas are exhibited in Table I. It will be

TABLE I

I		Octanol 1	PRIMARY
II		2 methyl heptanol 1	
III		3 methyl heptanol 1	
IV		4 methyl heptanol 1	
V		5 methyl heptanol 1	
VI		6 methyl heptanol 1	
VII		Octanol 2	SECONDARY
VIII		Octanol 3	
IX		Octanol 4	
X		3 methyl heptanol 2	
XI		4 methyl heptanol 2	
XII		5 methyl heptanol 2	
XIII		6 methyl heptanol 2	
XIV		2 methyl heptanol 3	
XV		4 methyl heptanol 3	
XVI		5 methyl heptanol 3	
XVII		6 methyl heptanol 3	
XVIII		2 methyl heptanol 4	
XIX		3 methyl heptanol 4	
XX		4 methyl heptanol 4	TERTIARY
XXI		3 methyl heptanol 3	
XXII		2 methyl heptanol 2	
XXIII		2 ethyl hexanol 1	PRIMARY

noted that of the twenty-three octyl alcohols examined, four were octanols, eighteen were heptanols, and one was a hexanol. The table, furthermore, gives a primary, secondary, and tertiary classification of the varieties prepared.

In making a study with the various octyl alcohols, an attempt was made at first to employ them in aqueous solution. Inasmuch as all of them are but slightly soluble in water, the writer resorted to a more satisfactory method. The various octyl alcohols were first dissolved in ethyl alcohol, 95 per cent, and such solutions in grain alcohol were employed as a starting-point for preparing exact dilutions or mixtures in water. A 1 per cent solution of octyl alcohol was usually prepared in 95 per cent ethyl alcohol, and this alcoholic solution could be more accurately measured and mixed with water than the straight octyl alcohols themselves. Previous investigations by Macht and Bloom (1921) and by Macht, Bloom, and Ting (1921) have shown that ethyl alcohol is comparatively very little toxic for various animals and that it had practically no effect in the dilutions employed in the present work, in which the strongest solutions of the octyl alcohols were 1:5,000 to 1 per cent ethyl alcohol. Control experiments made with ethyl alcohol were found to have no appreciable effect.

EXPERIMENTS ON CARASSIUS AUREUS

When goldfish are placed in dilute solutions of octyl alcohols, a pharmacological effect is usually soon noted. This is manifested in two ways: through the effect on the neuromuscular apparatus and through the effect on the respiratory center. The goldfish, when placed in sufficiently strong solutions, soon become more or less paralyzed and float on the side. The respiration is markedly slowed, and finally stopped completely as far as can be observed with the naked eye.

The results of this series of experiments are indicated in Table II. The end-point selected is complete paralysis of the respiratory movements of the gills. In the table are indicated the kinds of octyl alcohols used, their concentration in the solutions in which the fish were placed, and the time required for the complete paralysis of the respiration. When this end-point was reached, the fish were taken

out and placed in tap water, where most of them usually recovered, although they all remained for several days in a debilitated condi-

TABLE II

EXPERIMENTS ON *Carassius aureus* (GOLDFISH)

Paralysis of respiratory center and neuromuscular apparatus

Number	Octyl Alcohol	Time	Concentration Required	
I	Octanol 1	11 min.	1:15,000	PRIMARY
II	2 methyl heptanol 1	24 min.	1:15,000	
III	3 methyl heptanol 1	2 hrs.	1:15,000	
IV	4 methyl heptanol 1	42 min.	1:15,000	
V	5 methyl heptanol 1	25 min.	1:15,000	
VI	6 methyl heptanol 1	16 min.	1:15,000	
VII	Octanol 2	18 min.	1:15,000	SECONDARY
VIII	Octanol 3	44 min.	1:15,000	
IX	Octanol 4	50 min.	1:15,000	
X	3 methyl heptanol 2	4 hrs.	1:5,000	
XI	4 methyl heptanol 2	37 min.	1:15,000	
XII	5 methyl heptanol 2	45 min.	1:10,000	
XIII	6 methyl heptanol 2	30 min.	1:10,000	
XIV	2 methyl heptanol 3	13 min.	1:10,000	
XV	4 methyl heptanol 3	11 min.	1:10,000	
XVI	5 methyl heptanol 3	23 min.	1:10,000	
XVII	6 methyl heptanol 3	28 min.	1:10,000	
XVIII	2 methyl heptanol 4	30 min.	1:10,000	
XIX	3 methyl heptanol 4	40 min.	1:10,000	
XX	4 methyl heptanol 4	6 min.	1:5,000	TERTIARY
XXI	3 methyl heptanol 3	11 min.	1:5,000	
XXII	2 methyl heptanol 2	20 min.	1:5,000	
XXIII	2 ethyl hexanol 1	21 min.	1:15,000	PRIMARY

tion; some died. It will be noted that the concentrations of octyl alcohols employed were not the same in all cases. The primary alcohols were the most toxic, and various others also required a concentration of 1:15,000 for convenient experimentation. Other alcohols were used in concentrations of 1:10,000, and a third group was studied in solutions of 1:5,000. Beginning with the most toxic, the relative toxicity of the various octyl alcohols for the goldfish was displayed in the following order:

Concentration 1:15,000

I>VI>VII>XXIII>II>V>XIII>XI>IV>VIII>XII>IX>III

Concentration 1:10,000

XV>XIV>XVI>XVII>XVIII>XIX

Concentration 1:5,000

XXII>XXI>XX>X

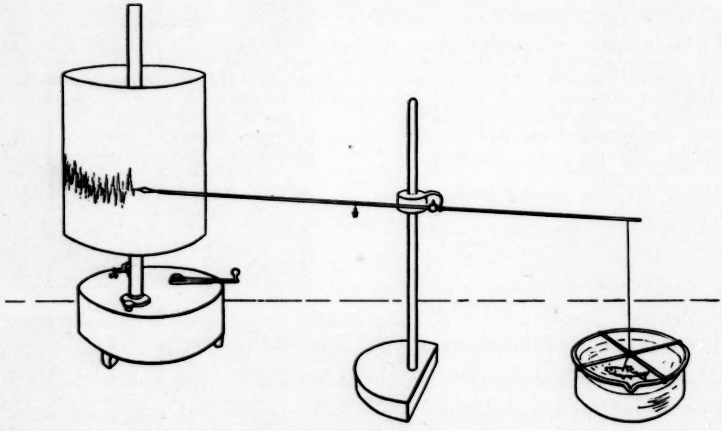


FIG. 1.—Ichthyometer

In addition to the data exhibited in Table II, giving the time of respiratory paralysis, the author performed an interesting series of experiments on goldfish by means of an apparatus described by Warren P. Spencer under the name of ichthyometer (Spencer, 1929) The set-up employed for the pharmacological work, illustrated in Figure 1, is a modification of that described by Spencer. A round

bowl is filled with water to the depth of 1½ inches. Over its top an arrangement of metal strips is so placed that the aperture made at their crossing is directly over the center of the bowl. A silk thread is attached just behind the dorsal fin of the fish by means of a small needle and passed through the aperture. It is then tied to one arm of a light lever, the other arm of which is slightly weighted, and allowed to trace the movements of the fish on a slowly revolving kymograph. By employment of a round bowl, not too wide and with a depth of water not too great, the movements of the fish are allowed the same maximum in all directions; and in this way a convenient ichthyogram for tracing can be obtained. The ichthyograms shown here illustrate well the difference in degree of toxicity between a number of primary, secondary, and tertiary octyl alcohols, as well as the effect of several combinations. These tracings indicate the onset of muscular paralysis in the fish. It will be noted in Figure 2 that ethyl alcohol in concen-

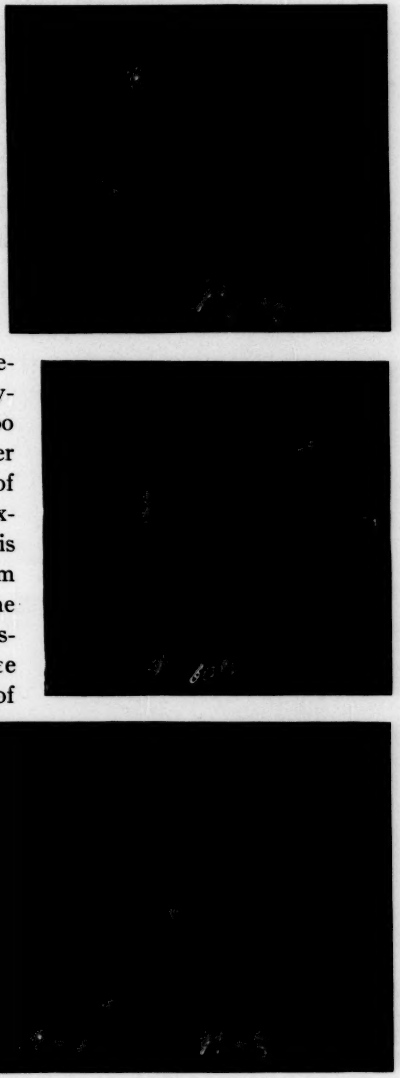


FIG. 2

FIG. 2.—Ichthyograms. With different concentrations of ethyl alcohol.

FIG. 3.—Ichthyograms. *A*, octyl alcohol I; *B*, octyl alcohol XX; *C*, octyl alcohol XII.

trations of 1:250, and even 1:50, is very slightly toxic. A concentration of 1:33 is required to finally paralyze the movements of the fish. On the other hand, the octyl alcohols promptly produce paralysis of the movements, and also what may be called "anesthetic effect," because the fish does not respond with a reflex movement to the touch. In Figure 3 it will be noted that primary octyl alcohol I was more active than secondary octyl alcohol XII, and the latter was more poisonous than the tertiary octyl XX. Figure 4 shows the results following use of various combinations, some effecting potentiation and others producing antagonistic action.

EXPERIMENTS ON DIEMYCTYLUS VIRIDESCENS

These animals were also studied by placing them in solutions of various octyl alcohols in water, as in case of goldfish. With the *Diemyctylus viridescens*, however, the end-point selected was the anesthesia of the tail or the failure of the animal to respond by a reflex when the tail was touched or lightly pinched. The results obtained in these experiments are illustrated in Table III. It will be noted that here again solutions of

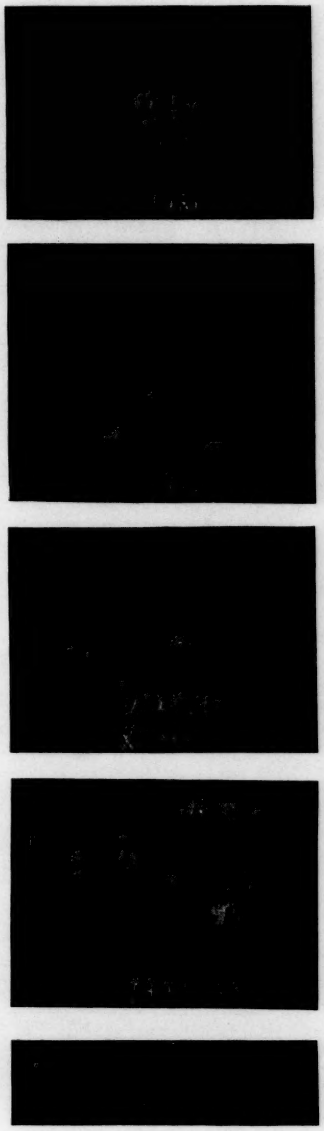


FIG. 4

FIG. 4.—Ichthyograms. *A*, octyl alcohols I and XII; *B*, octyl alcohols I and XX; *C*, octyl alcohols XII and XX; *D*, octyl alcohols I and XII and XX in ratio of 1:2:2.

TABLE III

EXPERIMENTS ON *Diemyctylus viridescens*

Anesthesia of tail

Number	Octyl Alcohol	Time	Concentration Required	
I	Octanol 1	2 min.	1:10,000	PRIMARY
II	2 methyl heptanol 1	3 min.	1:10,000	
III	3 methyl heptanol 1	4 min.	1:10,000	
IV	4 methyl heptanol 1	4 min.	1:10,000	
V	5 methyl heptanol 1	2½ min.	1:10,000	
VI	6 methyl heptanol 1	2 min.	1:10,000	
VII	Octanol 2	6 min.	1:10,000	SECONDARY
VIII	Octanol 3	7 min.	1:10,000	
IX	Octanol 4	8 min.	1:10,000	
X	3 methyl heptanol 2	2 hrs.	1:10,000	
XI	4 methyl heptanol 2	17 min.	1:10,000	
XII	5 methyl heptanol 2	27 min.	1:10,000	
XIII	6 methyl heptanol 2	20 min.	1:10,000	
XIV	2 methyl heptanol 3	25 min.	1:10,000	
XV	4 methyl heptanol 3	43 min.	1:10,000	
XVI	5 methyl heptanol 3	50 min.	1:10,000	
XVII	6 methyl heptanol 3	55 min.	1:10,000	
XVIII	2 methyl heptanol 4	57 min.	1:10,000	
XIX	3 methyl heptanol 4	60 min.	1:10,000	
XX	4 methyl heptanol 4	5 min.	1:5,000	TERTIARY
XXI	3 methyl heptanol 3	8 min.	1:5,000	
XXII	2 methyl heptanol 2	9 min.	1:5,000	
XXIII	2 ethyl hexanol 1	3 min.	1:15,000	PRIMARY

different concentration had to be employed in the various experiments. The relative toxicity of the various octyl alcohols for the *Diemyctylus viridescens* was displayed in the following order:

I>VI>V>II>XXIII>III>IV>VII>VIII>IX>
XI>XIII>XIV>XII>XV>XVI>XVII>
XVIII>XIX>XX>XXI>XXII>X

EFFECT OF COMBINATIONS

One of the most interesting and most puzzling phenomena of pharmacology is that of synergism. By this is meant the combined

TABLE IV

EXPERIMENTS ON *Carassius aureus*

Effect of combinations

Alcohol	Concentration	Paralysis of Respiration	Anesthesia and Loss of Equilibrium
Octyl I	1:15,000	12 min.	2 min.
Octyl VI	1:15,000	16 min.	2 min.
Octyl VII	1:15,000	17 min.	6 min.
Octyl XI	1:10,000	17 min.	7 min.
Octyl XX	1: 8,000	10 min.	5 min.
Octyls I and VI, equal parts*		7 min.	1 min.
Octyls I and VII, equal parts		9 min.	3 min.
Octyls VII and XI, equal parts		10 min.	3½ min.
Octyls I and XX, equal parts		6½ min.	2½ min.
Octyls VII and XX, equal parts		5½ min.	2 min.

* Equal parts of octyl alcohol, of one half of the concentrations given above, are mixed together in each case.

effect of two or more drugs administered simultaneously or closely following each other, an effect which is not equivalent to the simple arithmetical summation of the effects produced by the individual components. The whole phenomenon is discussed by the author at length in another paper (Macht, 1929), where it was shown that synergistic effects can be produced not only by combinations of two different drugs but even by stereoisomers. In the present research, several of the octyl alcohols were examined on goldfish both individually and in combination with each other, and the results obtained are shown in Table IV. On comparing the results obtained from these experiments with those in Table III, it will be noted

that combinations of various octyl alcohols yielded some striking synergistic effects.

DISCUSSION

An analysis and careful study of all the data obtained above lead to a number of interesting conclusions. In the first place, it is quite evident that the various octyl alcohols, although of identical empirical composition and differing from each other only in the relative positions of the hydroxyl and methyl groups, produce pharmacological effects of very different degrees. In the second place, an examination of the data obtained both with *Carassius aureus* and with *Diemyctylus viridescens*, broadly speaking, reveals that the primary octyl alcohols are more toxic than the secondary, and the secondary octyl alcohols are more toxic than the tertiary. In the third place, and from the pharmacological point of view of even greater consequence, is the fact that this relationship between the primary, secondary, and tertiary alcohols in respect to their toxicity is not an absolute one and that the exceptions to the rule are of extreme importance. Thus, for instance, octyl alcohol X does not fit into this scheme at all, and that is true of a number of other octyl alcohols. In the fourth place, the data given above exhibit a phenomenon which is well known to every pharmacologist but should be emphasized for the benefit of other biologists, namely, that the same drugs need not and usually do not act in the same way on different animals. The relative toxicity of octyl alcohols for *Carassius aureus* is not at all the same as the relative toxicity of those alcohols for *Diemyctylus viridescens*. Lastly, it will be seen from a study of the combinations that mixtures of two or more octyl alcohols produce pharmacological reactions which are not of a merely additive character but are synergistic, that is, the two components potentiate or otherwise affect the action of each other so as to produce unexpected results. These conclusions, drawn from the experimental data adduced above, cannot be too strongly emphasized in connection with studies of the relationship between chemical structure and pharmacological action of drugs and chemicals. The author has pointed out elsewhere (Macht, 1927) that it is extremely dangerous to make hasty generalizations in this field and that any relationship which one may endeavor to trace between

the structure and the physiological action of a chemical must be based on actual experiment.

SUMMARY

1. The pharmacological and toxicological action of twenty-three octyl alcohols was studied on *Carassius aureus* and *Diemyctylus viridescens*.

2. The various chemical structural isomers of octyl alcohol produce different degrees of pharmacodynamic reaction.

3. Generally speaking, the relative toxicity of primary octyl alcohols is greater than that of the secondary, and that of the secondary greater than that of the tertiary, but to this rule there are important exceptions.

4. The relative pharmacodynamic reactions of the various isomeric octyl alcohols for the two species of animals studied were very different.

5. The action of various combinations of the octyl alcohols produced synergistic effects.

6. In tracing the relationships in chemical structure and physiological action, all generalizations not based on actual experimental data are hazardous and should be discouraged.

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